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(54) Title: AGONIST PEPTIDE DIMERS

(57) Abstract

The present invention is directed to the dimerization of agonists and antagonists of cell surface receptors and particularly to peptide dimers which behave as cell surface receptor agonists in their dimeric form. Such receptors belong to the dimerization-mediated activation class often observed among receptors for growth and differentiation factors. The agonists of this class of receptors is understood to effect dimerization of the receptor and thus signal initiation. The present invention exemplifies dimers of erythropoietin (EPO) agonists and antagonists comprising a core amino acid sequence of $X_3X_4X_5GPX_6TWX_7X_8$ (SEQ ID NO: 1) wherein each amino acid is indicated by standard one letter abbreviation; X_3 can be C, A, α -amino- γ -bromobutyric acid or Hoc; X_4 can be R, H, L or W; X_5 can be M, F, or I; X_6 is independently selected from any one of the 20 genetically coded L-amino acids or the stereoisomeric D-amino acids; X_7 can be D, E, I, L or V; and X_8 can be C, A, α -amino- γ -bromobutyric acid or Hoc, provided that either X_1 or X_8 is C or Hoc.

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GA	Gabon			VN	Viet Nam

1 AGONIST PEPTIDE DIMERSFIELD OF THE INVENTION

5 The present invention is directed to the dimerization of agonists and antagonists of cell surface receptors and particularly to peptide dimers which behave as cell surface receptor agonists in their dimeric form. Such receptors belong to the
10 dimerization-mediated activation class often observed among receptors for growth and differentiation factors. The agonists of this class of receptors is understood to effect dimerization of the receptor and thus signal initiation. The present invention exemplifies dimers of erythropoietin (EPO) agonists and antagonists comprising
15 a core amino acid sequence of $X_3X_4X_5GPX_6TWX_7X_8$ (SEQ ID NO: 1) wherein each amino acid is indicated by standard one letter abbreviation; X_3 can be C, A, α -amino- γ -bromobutyric acid or Hoc; X_4 can be R, H, L or W; X_5 can be M, F, or I; X_6 is independently selected from any one of the 20 genetically coded L-amino acids or the stereoisomeric D-amino acids; X_7 can be D, E, I, L or V; and X_8 can be C, A, α -amino- γ -bromobutyric acid or Hoc, provided that either X_3 or X_8 is C or Hoc.

25 BACKGROUND OF THE INVENTION

30 Erythropoietin (EPO) is a glycoprotein hormone with an approximate molecular weight of 34,000 daltons. The primary role of EPO, which is synthesized in the kidneys of mammals, is to stimulate mitotic cell division and differentiation of erythrocyte precursor cells. As a result, EPO acts to stimulate and to

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1 regulate the production of erythrocytes. Erythrocytes,
and the hemoglobin contained therein, play a central
role in supplying oxygen to the body. Thus, the
5 stimulation of erythrocyte production is able to
increase the oxygen-carrying capacity of the blood.

10 During normal conditions, EPO is present in
very low concentrations in plasma. Under hypoxic
conditions, the amount of EPO in the circulation is
increased in response to reduced O₂ blood levels.

15 Hypoxia may be caused from various conditions including
the loss of large amounts of blood, destruction of red
blood cells by over-exposure to radiation or
chemotherapeutic agents, reduction in oxygen intake due
to high altitudes or prolonged unconsciousness, or by
various forms of anemia. As the hypoxic condition
diminishes, the amount of EPO produced subsequently
diminishes.

20 Because of the essential role of EPO in red
blood cell formation, the hormone is useful in both the
diagnosis and the treatment of blood disorders
characterized by low or defective red blood cell
production. Recent studies provide a basis for the
efficacy of EPO therapy in a variety of disease states,
disorders, and states of hematologic irregularity,
25 including: beta-thalassemia (See, Vedovato et al.
(1984) Acta Haematol. 71:211-213); cystic fibrosis
(See, Vichinsky et al. (1984) J. Pediatric 105:15-21);
pregnancy and menstrual disorders (See, Cotes et al.
30 (1983) Brit. J. Obstet. Gynaecol. 90:304-311); early
anemia of prematurity (See, Haga et al. (1983) Acta
Pediatri. Scand. 72:827-831); spinal cord injury (See,

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1 Claus-Walker et al. (1984) Arch. Phys. Med. Rehabil.
 65:370-374); space flight (See, Dunn et al. (1984) Eur.
 J. Appl. Physiol. 52:178-182); acute blood loss (See,
 5 Miller et al. (1982) Brit. J. Haematol. 52:545-590);
 aging (See, Udupa et al. (1984) J. Lab. Clin. Med.
 10 103:574-588); various neoplastic disease states
 accompanied by abnormal erythropoiesis (See, Dainiak et
 al. (1983) Cancer 5:1101-1106); and renal insufficiency
 (See, Eschbach et al. (1987) N. Eng. J. Med. 316:73-78).

10 Although purified, homogenous EPO has been
 characterized, little is known about the mechanism of
 EPO-induced erythroblast proliferation and
 differentiation. The specific interaction of EPO with
 15 progenitor cells of immature red blood cells, platelets,
 and megakaryocytes has not been described. This is due
 in part, to the small number of surface EPO receptor
 molecules on normal erythroblasts and on the
 erythroleukemia cell lines. See Krantz and Goldwasser
 20 (1984) Proc. Natl. Acad. Sci. USA, 81:7574-7578; Branch
 et al. (1987) Blood 69:1782-1785; Mayeux et al. (1987)
 25 FEBS Letters 211:229-233; Mufson and Gesner (1987) Blood
 69:1485-1490; Sakaguchi et al. (1987) Biochem. Biophys.
 Res. Commun. 146:7-12; Sawyer et al. (1987) Proc. Natl.
 Acad. Sci. USA 84:3690-3694; Sawyer et al. (1987) J.
 Biol. Chem. 262:5554-5562; and Todokoro et al. (1988)
 30 Proc. Natl. Acad. Sci. USA 84:4126-4130. The DNA
 sequences and encoded peptide sequences for murine and
 human EPO receptor proteins have been described. See,
 D'Andrea et al. PCT Patent Publication No. WO 90/08822
 (published 1990).

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1 The EPO-receptor (EPO-R) belongs to the class
of growth-factor-type receptors which are activated by a
ligand-induced protein dimerization. Other hormones and
5 cytokines such as human growth hormone (hGH),
granulocyte colony stimulating factor (G-CSF), epidermal
growth factor (EGF) and insulin can cross-link two
receptors resulting in juxtaposition of two cytoplasmic
tails. Many of these dimerization-activated receptors
10 have protein kinase domains within the cytoplasmic tails
that phosphorylate the neighboring tail upon
dimerization. While some cytoplasmic tails lack
intrinsic kinase activity, these function by association
with protein kinases. The EPO receptor is of the latter
type. In each case, phosphorylation results in the
15 activation of a signaling pathway.

20 In accordance with the present invention, it
has been discovered that the dimerization of peptide
agonists and antagonists of dimerization-mediated
receptors, such as EPO-R, increase the biological
efficacy relative to the biological activity of the
25 'monomeric' agonists and alters the properties of the
antagonists such that, these dimers function as
agonists, exhibiting biological activity.

30 SUMMARY OF THE INVENTION

35 In a first embodiment, the present invention
is directed to peptide dimers which behave as cell-
surface receptor agonists, dimers which exhibit binding
and signal initiation of growth factor-type receptors.
In one embodiment, the present invention provides
peptide dimers which behave as EPO agonists. These

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1 dimers have two 'monomeric' peptide units of 10 to 40 or
more amino acids, preferably 14 to about 20 residues in
length, comprising a core amino acid sequence of
5 $X_1X_2X_3GPX_6TWX_7X_8$ (SEQ ID NO: 1) where each amino acid is
indicated by standard one letter abbreviation; X_1 can be
C, A, α -amino- γ -bromobutyric acid, or Hoc, where Hoc is
homocysteine; X_2 can be R, H, L, or W; X_3 can be M, F,
or I; X_6 is independently selected from any one of the
10 20 genetically coded L-amino acids or the stereoisomeric
D-amino acids; X_7 can be D, E, I, L, or V; and X_8 can be
C, A, α -amino- γ -bromobutyric acid, or Hoc, where Hoc is
homocysteine, provided that either X_1 , or X_8 is C or Hoc.
Preferably, the monomeric peptide unit of the dimer
15 comprises a core sequence $YX_2X_3X_4X_5GPX_6TWX_7X_8$ (SEQ ID
NO: 2) where each amino acid is indicated by standard
one letter abbreviation; each X_2 and X_6 is independently
selected from any one of the 20 genetically coded L-
amino acids; X_3 can be C, A, α -amino- γ -bromobutyric
20 acid, or Hoc, where Hoc is homocysteine; X_4 can be R, H,
L, or W; X_5 can be M, F, or I; X_7 can be D, E, I, L, or
V; and X_8 can be C, A, α -amino- γ -bromobutyric acid, or
Hoc, where Hoc is homocysteine, provided that either X_3 ,
or X_8 is C or Hoc.

25 More preferably, the monomeric peptide unit of
the dimer comprises a core sequence of amino acids
 $X_1YX_2X_3X_4X_5GPX_6TWX_7X_8X_9X_{10}X_{11}$ (SEQ ID NO: 3), where each
amino acid is indicated by standard one letter
abbreviation; each X_1 , X_2 , X_3 , X_4 , X_5 , X_6 , X_7 , X_8 , X_9 , X_{10} , and X_{11} is
30 independently selected from any one of the 20
genetically coded L-amino acids; X_1 can be C, A, α -
amino- γ -bromobutyric acid, or Hoc, where Hoc is

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1 homocysteine; X₄ can be R, H, L, or W; X₅ can be M, F, or I; X₇ can be D, E, I, L or V; and X₈ can be C, A, α-amino-γ-bromobutyric acid, or Hoc, where Hoc is homocysteine, provided that either X₃ or X₈ is C or Hoc.

5 In a more preferred embodiment, both X₃ and X₈ are C and thus, the monomeric peptide unit of the dimer comprises a core sequence of amino acids

10 X₁YX₂CX₄X₅GPX₆TWX₇CX₉X₁₀X₁₁ (SEQ ID NO: 4). More preferably, the monomeric peptide unit comprises a core sequence of amino acids X₁YX₂CX₄X₅GPX₆TWX₇CX₉X₁₀X₁₁ (SEQ ID NO: 5), where X₄ can be R or H; X₅ can be F or M; X₆ can be I, L, T, M, or V; X₇ is D or V; X₉ can be G, K, L, Q, R, S, or T; and X₁₀ can be A, G, P, R, or Y. In a most preferred embodiment, the monomeric peptide unit of the dimer will comprise a core sequence of amino acids X₁YX₂CX₄X₅GPX₆TWX₇CX₉X₁₀X₁₁ (SEQ ID NO: 6), where X₁ can be D, E, L, N, S, T, or V; X₂ can be A, H, K, L, M, S, or T; X₄ is R or H; X₅ can be K, R, S, or T; and X₁₀ is P. Particularly preferred monomeric peptide units of the dimers include:

20 GGLYLCRFGPVTWDCGYKGG (SEQ ID NO: 7);
GGTYSCHFGPLTWVCKPQGG (SEQ ID NO: 8);
GGDYHCRMGPLTWVCKPLGG (SEQ ID NO: 9);
VGNYMCHFGPITWVCRPGGG (SEQ ID NO: 10);
GGVYACRMGPITWVCSPLGG (SEQ ID NO: 11);
VGNYMAHMGPITWVCRPGG (SEQ ID NO: 12);
GGTYSCHFGPLTWVCKPQ (SEQ ID NO: 13);
GGLYACHMGPMTWVCQPLRG (SEQ ID NO: 14);
TIAQYICYMGPETWECRSPKA (SEQ ID NO: 15);
YSCHFGPLTWVCK (SEQ ID NO: 15);
YCHFGPLTWVC (SEQ ID NO: 17); and

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1

SCHFGPLTWVCK

(SEQ ID NO: 18).

Other particularly preferred monomeric peptide units of the present dimers include peptides comprising a core sequence of the formula $(AX_2)_nX_3X_4X_5GPX_6TWX_7X_8$ (SEQ ID NO: 19) wherein X_2 through X_8 are as previously defined herein (SEQ ID NO: 2), n is 1 or 0 and A is any one of the naturally occurring L-amino acids except Y (tyrosine); n is defined herein as the number of occurrences of (AX_2) which can be 1 or none in the core sequence. When (AX_2) is present, i.e. when $n = 1$, A is not tyrosine and A is not any non-naturally occurring aromatic amino acid analog. Such monomeric peptide units of the dimers of this invention can be prepared by truncating the peptides of Fig. 9, for example, from the N-terminus to delete the Y, tyrosine residue in SEQ ID NOS. 21 - 93. Such monomeric peptides can also be prepared by substitution of Y in position A in the peptides of Fig. 9.

20

In accordance with the present invention the monomeric units of the dimers can be the same or different.

25

In a preferred embodiment polyethylene glycol (PEG) is employed as a linker to form the dimeric peptides of the present invention through a covalent bond.

30

In another embodiment, the present invention is directed to pharmaceutical compositions comprising at least one dimer peptide of the invention and a pharmaceutical carrier.

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1 In a further embodiment, the present invention
provides a method for therapeutically treating a mammal
having a condition resulting from a hormone or growth
factor deficiency by administration of at least one of
5 the dimer peptides of the present invention.

10 In a still further embodiment, a method for
therapeutically treating a mammal having a condition
resulting from a deficiency of EPO or from reduced
levels of blood oxygen caused by a decrease in
erythrocyte number is provided.

15 In another embodiment of this invention, a
method is provided for preparing agonists of cell-
surface receptors wherein agonists of the class of cell-
surface or dimerization-mediated receptors are dimerized
to enhance the in vitro or in vivo biological activity
of the cell-surface receptor relative to the monomeric
agonists from which the dimer is derived. This method is
also directed to the preparation of agonists of such
20 growth-factor-type receptors by dimerizing antagonists
of these receptors; the dimerized 'antagonists' thereby
exhibit agonist biological activity in vitro and in
vivo. In a preferred embodiment, the present method is
directed to the preparation of EPO-R dimer agonists from
monomeric EPO-R antagonists.

25

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows a major peak, with a retention
time of 37 minutes, of the dimerized EPO peptide,
GGTYSCHFGPLTWVCKPQGG (SEQ ID NO: 8)

30

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Fig. 2 shows a major peak, with a retention time of 48 minutes, following purification of the dimerized EPO peptide, (SEQ ID NO: 8).

5

Fig. 3 depicts the MALDI-TOF mass spectral analysis of the dimerized peptides, including peptide (SEQ ID NO: 8), GGTYSCHFGPLTWVCKPQ (SEQ ID NO: 13) and SCHFGPLTWVCK (SEQ ID NO: 18).

10

Fig. 4 shows the SDS-PAGE analysis of DPDPB crosslinking of EPO binding protein (EBP) in the presence and absence of EPO agonist peptides.

15

Fig. 5 demonstrates equilibrium EPO binding to immobilized EPO binding protein. Panel A represents the equilibrium binding data and Panel B (inset) is the linear transformation (Scatchard) of the data set in Panel A.

20

Fig. 6 depicts the results of a competitive binding assay run on the EPO agonist peptide (SEQ ID NO: 8) in competitive binding with [¹²⁵I]EPO to EBP beads (Panel A); and EPO responsive cell proliferation studies in FDC-P1 derived cell lines containing either a human (Panel B) or murine EPO receptor (Panel C).

25

Fig. 7 is a graphic representation of the results of the exhypoxic mouse bioassay; stimulation of the incorporation of [⁵⁵Fe] into nascent red blood cells by EPO, peptide (SEQ ID NO: 8) (Panel A) and peptide (SEQ ID NO: 8) dimer (Panel B).

30

Fig. 8 demonstrates the effect of PEG dimerization of peptide (SEQ ID NO: 18) activity in EPO responsive cell proliferation studies in FDC-P1 derived cell lines containing a human EPO receptor.

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1 Fig. 9 provides the sequences of
representative monomeric peptides of the present
invention.

5 DETAILED DESCRIPTION OF THE INVENTION

10 The present invention is directed to peptide
dimers which behave as cell surface receptor agonists,
dimers which exhibit binding and signal initiation of
growth-factor-type receptors. Sometimes called cell-
surface receptors, growth-factor-type receptors or
15 dimerization-mediated activator-receptors, these are a
class of molecules which are understood to be activated
by ligand-induced or ligand stabilized dimerization.
Agonists of such receptors typically include large
polypeptide hormones including the cytokines, insulin
20 and various other growth or differentiation factors.
The agonists are understood to induce dimerization of
the receptor and thereby effect signal initiation. Such
agonists are believed to effectively cross-link two
receptors resulting in the repositioning of cytoplasmic
25 tails which may directly or indirectly effect
phosphorylation of the cytoplasmic tails and activation
of a signaling pathway.

30 The present invention specifically includes
those molecules which behave as agonists of cell-surface
receptors when dimerized in accordance with this
invention. Such dimer agonists can include 'monomeric'
units which exhibit agonist or antagonist activity for
the related receptor molecule and may be the same or
different. The dimers are preferably peptides but may
alternatively be small molecule pharmacophores. These
molecules when dimerized exhibit agonist activity of

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1 cell-surface receptors in vitro and in vivo. Such
receptors include, for example, EPO, GM-CSF, G-CSF, M-
CSF, GH, EGF, PDGF, VEGF, Insulin and FGF. Other
5 receptors which are activated by heterodimerization or
multimerization may also be subject to activation by
this mechanism including, IL-3, IL-5, IL-6, IL-2 and
TPO. The dimers of the present invention have two
10 'monomeric' peptide units of 10 to 40 or more amino
acids, preferably 14 to about 20 amino acid residues in
length. In a preferred embodiment, these monomeric
peptide units comprise a core sequence of amino acids
X₃X₄X₅GPX₆TWX₇X₈ (SEQ ID NO: 1) where each amino acid is
indicated by standard one letter abbreviation; X₃ can be
15 C, A, α -amino- γ -bromobutyric acid, or Hoc, where Hoc is
homocysteine; X₄ can be R, H, L, or W; X₅ can be M, F,
or I; X₆ is independently selected from any one of the
20 20 genetically coded L-amino acids or the stereoisomeric
D-amino acids; X₇ can be D, E, I, L, or V; and X₈ can be
C, A, α -amino- γ -bromobutyric acid, or Hoc, where Hoc is
homocysteine, provided that either X₃ or X₈ is C or Hoc.
Preferably, the monomeric peptide unit of the dimer
25 comprises a core sequence YX₂X₃X₄X₅GPX₆TWX₇X₈ (SEQ ID
NO: 2) where each amino acid is indicated by standard
one letter abbreviation; each X₂ and X₈ is independently
selected from any one of the 20 genetically coded L-
amino acids; X₃ can be C, A, α -amino- γ -bromobutyric
acid, or Hoc, where Hoc is homocysteine; X₄ can be R, H,
L, or W; X₅ can be M, F, or I; X₇ can be D, E, I, L, or
V; and X₈ can be C, A, α -amino- γ -bromobutyric acid, or
Hoc, where Hoc is homocysteine, provided that either X₃
30 or X₈ is C or Hoc.

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1 More preferably, the monomeric peptide unit of
the dimer comprises a core sequence of amino acids
 $X_1YX_2X_3X_4X_5GPX_6TWX_7X_8X_9X_{10}X_{11}$ (SEQ ID NO: 3), where each
5 amino acid is indicated by standard one letter
abbreviation; each X_1 , X_2 , X_3 , X_4 , X_5 , X_8 , and X_{11} is
independently selected from any one of the 20
genetically coded L-amino acids; X_3 can be C, A,
10 α -amino- γ -bromobutyric acid, or Hoc, where Hoc is
homocysteine; X_4 can be R, H, L, or W; X_5 can be M, F,
or I; X_8 can be D, E, I, L or V; and X_9 can be C, A,
 α -amino- γ -bromobutyric acid, or Hoc, where Hoc is
homocysteine, provided that either X_3 or X_8 is C or Hoc.

15 In a more preferred embodiment, both X_3 and X_8
will be C and thus, the monomeric peptide unit of the
dimer comprises a core sequence of amino acids
 $X_1YX_2CX_4X_5GPX_6TWX_7CX_9X_{10}X_{11}$ (SEQ ID NO: 4). More
preferably, the monomeric peptide unit comprises a core
sequence of amino acids $X_1YX_2CX_4X_5GPX_6TWX_7CX_9X_{10}X_{11}$ (SEQ
20 ID NO: 5), where X_4 can be R or H; X_5 can be F or M; X_8
can be I, L, T, M, or V; X_9 is D or V; X_{10} can be G, K,
L, Q, R, S, or T; and X_{11} can be A, G, P, R, or Y. In a
most preferred embodiment, the monomeric peptide unit of
the dimer comprises a core sequence of amino acids
 $X_1YX_2CX_4X_5GPX_6TWX_7CX_9X_{10}X_{11}$ (SEQ ID NO: 6), where X_1 can
25 be D, E, L, N, S, T, or V; X_2 can be A, H, K, L, M, S,
or T; X_4 is R or H; X_5 can be K, R, S, or T; and X_{10} is
P. Particularly preferred monomeric peptide units of
the present dimers include:

30 GGLYLCRFGPVTWDCGYKGG (SEQ ID NO: 7);
GGTYSCHFGPLTWVCKPQGG (SEQ ID NO: 8);

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1 GGDYHCRMGPLTWVCKPLGG (SEQ ID NO: 9);
 VGNYMCHFGPITWVCRPGGG (SEQ ID NO: 10);
 GGVYACRMGPITWVCSPLGG (SEQ ID NO: 11);
5 VGNYMAHMGPIWVCRPGG (SEQ ID NO: 12);
 GGTYSCHFGPLTWVCKPQ (SEQ ID NO: 13);
 GGLYACHMGPMTWVCQPLRG (SEQ ID NO: 14);
 TIAQYICYMGPETWECRSPSKA (SEQ ID NO: 15);
 YSCHFGPLTWVCK (SEQ ID NO: 16);
10 YCHFGPLTWVC (SEQ ID NO: 17); and
 SCHFGPLTWVCK (SEQ ID NO: 18).

The dimer peptides of the present invention exhibit increased biological potency in vitro and in vivo relative to the monomeric agonists from which the dimers are derived. Moreover, cell surface receptor antagonists can be 'converted' to cell surface receptor agonists in accordance with the present invention. Specifically, a cell surface receptor antagonist can be dimerized with PEG or another appropriate linker which permits mutual binding of the monomeric moieties with the receptors. As a result, the dimer exhibits effective binding to the target receptor and behaves as an agonist. Accordingly, the dimers of this invention demonstrate enhanced biological potency in vitro and in vivo relative to their monomeric forms.

The dimer peptides of the present invention bind to and biologically activate the cell surface receptor or otherwise behave as agonists and are preferably formed by employing polyethylene glycol as a linker between the monomeric peptide units described herein. While other conventional chemical systems can

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1 also be employed to form the dimer peptides of this
invention including using other known polymeric
compounds, pegylation is preferred.

5 The linking compounds of the present invention
include any molecule which covalently binds the
monomeric peptides at an appropriate distance or which
otherwise effects dimerization of the particular cell
surface receptor thereby initiating biological efficacy.

10 Starting with an appropriate synthetic
peptide, containing a free amino group or other reactive
site such as hydroxyls, carboxylic acids or sulfhydryls,
the peptide is added in excess to a reaction mixture
containing a corresponding reactive polymer. The
polymer can be of a repeating nature such as
15 polyethylene glycol, peptides, modified peptides or
peptide analogs. Alternatively, the peptide can be
dimerized on a small molecule scaffold such as activated
benodiazepins, oxazolones, azalactones, aminimides or
20 diketopiperazines. The most readily available linker of
variable distance are ones based on linear unbranched
polyethylene glycols.

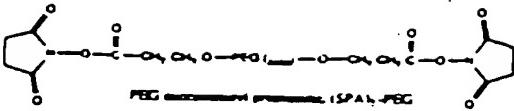
25 The following is a schematic of a preferred
preparatory methodology employing PEG succinimidyl
propionate as the linker between the monomer units of
the dimer peptides.

30

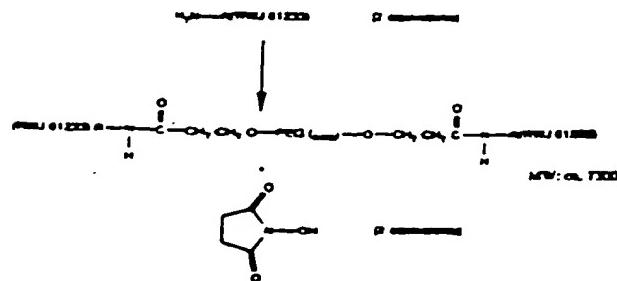
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Dimerization and especially pegylation in a head-to-head (amino to amino terminus) or head-to-tail (amino to carboxyl terminus) configuration is preferred relative to internal covalent binding of the monomeric peptides. The 'monomer' units of the dimer peptides of the present invention can be the same or different, although the same are preferred.

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The monomeric peptides which are used to form the dimers of the present invention can be prepared by classical chemical methods well known in the art. The standard methods include, for example, exclusive solid phase synthesis and recombinant DNA technology. See, e.g. Merrifield (1963) J. Am. Soc. 85:2149. Solid phase synthesis is typically commenced from the C-terminal end of the peptide using an α -amino protected resin. A suitable starting material can be prepared by attaching the required α -amino acid to a chloromethylated resin (such as BIO-BEADS SX-1, Bio Rad Laboratories, Richmond, CA), a hydroxymethyl resin, (described by Bodanszky et al. (1966) Chem. Ind.

- 16 -

1 (London) 38:1597) or a benzhydrylamine resin (described
by Pietta and Marshall (1970) Chem. Commun. 650).

5 The α -amino protecting groups are those known
to be useful in the art of stepwise synthesis of
peptides. Included are acyl type protecting groups
(e.g. formyl, trifluoroacetyl, acetyl), aromatic
urethane type protecting groups (e.g. benzyloxycarboyl
(Cbz) and substituted Cbz), aliphatic urethane
protecting groups (e.g., t-butyloxycarbonyl (Boc),
isopropylloxycarbonyl, cyclohexyloxycarbonyl) and alkyl
type protecting groups (e.g., benzyl and
triphenylmethyl). The preferred X-amino protecting
group is Fmoc. The side chain protecting group
(typically ethers, esters, trityl, PMC, and the like)
remains intact during coupling and is not split off
during the deprotection of the amino-terminus protecting
group or during coupling. The side chain protecting
group must be removable upon the completion of the
synthesis of the final peptide and under reaction
conditions that will not alter the target peptide.

10 The side chain protecting groups for Tyr
include tetrahydropyranyl, tert-butyl, trityl, benzyl,
Cbz, Z-Br-Cbz, and 2,5-dichlorobenzyl. The side chain
protecting groups for Asp include benzyl, 2,6-
dichlorobenzyl, methyl, ethyl, and cyclohexyl. The side
chain protecting groups for Thr and Ser include acetyl,
benzoyl, trityl, tetrahydropyranyl, benzyl, 2,6-
dichlorobenzyl, and Cbz. The side chain protecting
groups for Thr and Ser are benzyl. The side chain
protecting groups for Arg include nitro, Tosyl. (Tcs),
Cbz, adamantyloxycarbonyl mesitoylsulfonyl (Mts), or

- 17 -

1 Boc. The side chain protecting groups for Lys include Cbz, 2-chlorobenzylloxycarbonyl (2-Cl-Cbz), 2-bromobenzylloxycarbonyl (2-BrCbz), Tos, or Boc.

5 After removal of the α -amino protecting group, the remaining protected amino acids are coupled stepwise in the desired order. Each protected amino acid is generally reacted in about a 3-fold excess using an appropriate carboxyl group activator such as 2-(1H-benxotriazol-1-yl)-1,1,3,3tetramethyluronium
10 hexafluorophosphate (HBTU) or dicyclohexylcarbodiimide (DCC) in solution of methylene chloride (CH_2Cl_2), or dimethyl formamide (DMF) mixtures.

15 After the desired amino acid sequence has been completed, the desired peptide is decoupled from the resin support by treating the mixture with a reagent such as trifluoroacetic acid (TFA) or hydrogen fluoride (HF). These reagents not only cleave the peptide from the resin, but also cleave all remaining side chain protecting groups. When the chloromethylated resin is used, hydrogen fluoride treatment results in the formation of the free peptide acids. When the benzhydrylamine resin is used, hydrogen fluoride treatment results directly in the free peptide amide. Alternatively, when the chloromethylated resin is employed, the side chain protected peptide can be decoupled by treatment of the peptide resin with ammonia to give the desired side chain protected amide or with an alkylamine to give a side chain protected alkylamide or dialkylamide. Side chain protection is then removed in the usual fashion by treatment with hydrogen fluoride to give the free amides, alkylamides, or dialkylamides.

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1 These procedures can also be used to
synthesize peptides in which amino acids other than the
20 naturally occurring, genetically encoded amino acids
are substituted at one, two or more positions of any of
5 the compounds of the invention. For instance,
naphthylalanine can be substituted for tryptophan,
facilitating synthesis. Other synthetic amino acids
that can be substituted into the peptides of the present
10 invention include L-hydroxypropyl, L-3, 4-
dehydroxyphenylalanyl, δ amino acids such as L- δ -
hydroxylysyl and D- δ -methylalanyl, L- α -methylalanyl, β
amino acids, and isoquinolyl. D-amino acids and non-
naturally occurring synthetic amino acids can also be
incorporated into the peptides of the present invention.
15

15 In another embodiment of the present
invention, a method of enhancing the in vitro or in vivo
biological potency of a cell surface receptor agonist is
provided. This methodology is achieved by dimerizing
the receptor agonist with a linker molecule, such as
20 PEG, to form an appropriate spatial relationship between
the monomeric peptide units of the dimer and thereby
permitting each of the constituents of the dimers to
bind to their receptors to achieve enhanced biological
potency, i.e., to dimerize and thereby activate the
25 receptors to induce the relevant biological activity of
the particular cell-surface receptor, e.g. EPO-R.
Biological activity can be measured by the skilled
artisan in various in vitro and in vivo assays and as
demonstrated in the examples of the present invention.
30

30 The peptide or molecule with binding affinity
for a given receptor will have increased conformational

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1 flexibility leading to fewer barriers to effective
receptor interaction and subsequently receptor
activation. This is also indicated for molecules which
5 can bind but not activate a receptor subtype in that
such molecules can become more effective inhibitors of
ligand binding.

The present invention further provides a method for altering a cell-surface receptor antagonist, a molecule exhibiting receptor binding but no biological activity, to behave as a cell-surface receptor agonist in vitro or in vivo. This method is achieved by dimerizing the antagonist molecule with an appropriate linker molecule such as PEG, other polymerized molecules or a peptide. In a preferred embodiment, an EPO antagonist, i.e. a peptide exhibiting receptor binding but no biological EPO activity can be altered by dimerization to obtain a dimer which behaves as an EPO receptor agonist. Thus, for example, in the case of EPO-R these include the monomeric peptide units of the present dimers comprising a core sequence of general formula $(AX_2)_nX_3X_4X_5GPX_6TWX_7X_8$ (SEQ ID NO: 19) wherein X_2 through X_8 are as previously defined herein, in (SEQ ID NO: 2), n is 1 or 0 and A is any one of the naturally occurring L-amino acids except Y (tyrosine); n is defined herein as the number of occurrences of (AX_2) which can be 1 or none in the core sequence. When X_2 is present, i.e., when $n = 1$, A is not tyrosine and A is not any non-naturally occurring aromatic amino acid analog. Such monomeric peptide units of the dimers of this invention can be prepared by truncating the peptides of Fig. 9, for example, from the N-terminus to

-20-

1 delete the Y, tyrosine residue in SEQ ID NOS. 21 - 93.
Such monomeric peptides can also be prepared by
substitution of Y in the peptides of Fig. 9.

5 These molecules, demonstrate only binding
activity in their 'monomeric' form, but exhibit agonist
activity after dimerization with a linking compound such
as PEG. Accordingly, the present method comprises
identifying a monomeric peptide as herein defined which
does not demonstrate biological activity and dimerizing
10 that antagonist in accordance with the present invention
to obtain a cell-surface-receptor agonist i.e., in
dimeric form. Contacting the appropriate cell-surface
receptor with the thus formed dimer activates, i.e.
15 dimerizes such receptors and thus induces biological
activity of the receptor. Such monomeric units as
shown in Fig. 9 can be truncated from the N-terminus
such as SCHFGPLTWVCK (SEQ ID NO: 18) to eliminate the
tyrosine residue at position A of the formula
20 $(AX_2)_nX_3X_4X_5GPX_6TWX_7X_8$ (SEQ ID NO: 19) or merely
substituted with any of the remaining 19 naturally
occurring amino acids or with other than a non-naturally
occurring aromatic amino acid analog. In accordance
25 with the present invention it has been determined that
the tyrosine residue at position A of the foregoing
formula is critical to biological activity of the
monomer peptide. Deletion or substitution of the
tyrosine eliminates biological activity. When dimerized
however the entity exhibits enhanced biological
activity.

30 For example, tyrosine (Y) substituted in the
formula $YX_2X_3X_4X_5GPX_6X_8$ (SEQ ID NO: 2) by p-

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1 iodohydroxyphenylalanine, p-fluorohydroxyphenylalanine,
p-amino-hydroxyphenylalanine act as EPO-R monomer
agonists but substitution with threonine or alanine for
5 tyrosine at position Y causes the monomer peptide to act
as an EPO-R antagonist. However, when dimerized in
accordance with the present invention, such dimers
behave as EPO-R agonists. The monomeric peptide units
identified at Fig. 9, for example, behave as EPO-R
10 antagonists in the absence of tyrosine at position Y of
the formula above. When such antagonists are dimerized,
the dimer behaves as an EPO-R agonist.

15 In a further embodiment of the present
invention, pharmaceutical compositions comprising at
least one of the dimers of this invention can be
employed to therapeutically treat disorders resulting
from deficiencies of biological factors such as EPO, GH,
GM-CSF, G-CSF, EGF, PDGF, VEGF, insulin, FGF and the
like. These pharmaceutical compositions may contain
20 buffers, salts and other excipients to stabilize the
composition or assist in the delivery of the dimerized
molecules.

25 In a preferred embodiment, the present
invention provides a method for treating disorders
associated with a deficiency of EPO. The method is
accomplished by administering at least one of the dimers
identified herein for a time and under conditions
sufficient to alleviate the symptoms of the disorder,
i.e. sufficient to effect dimerization or biological
activation of EPO receptors. In the case of EPO such
30 methodology is useful in the treatment of end-stage
renal failure/dialysis; anemia, especially associated

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1 with AIDS or chronic inflammatory diseases such as
rheumatoid arthritis and chronic bowel inflammation;
auto-immune disease; and for boosting the red blood cell
count of patient when necessary, e.g. prior to surgery
5 or as pretreatment to transfusion. The dimers of the
present invention which behave as EPO agonists can be
used to activate megakaryocytes.

10 Since EPO has been shown to have a mitogenic
and chemotactic effect on vascular endothelial cells as
well as an effect on central cholinergic neurons (see,
e.g., Amagnostou et al. (1990) Proc. Natl. Acad. Sci.
USA 87:597805982 and Konishi et al. (1993) Brain Res.
15 609:29-35), the compounds of this invention can also be
used to treat a variety of vascular disorders, such as
promoting wound healing, growth of collateral coronary
blood vessels (such as those that may occur after
myocardial infarction), trauma, and post vascular graft
treatment, and a variety of neurological disorders,
generally characterized by low absolute levels of acetyl
20 choline or low relative levels of acetyl choline as
compared to other neuroactive substances e.g.,
neurotransmitters.

25 Accordingly, the present invention includes
pharmaceutical compositions comprising, as an active
ingredient, at least one of the peptide dimers of the
present invention in association with a pharmaceutical
carrier or diluent. The dimers of this invention can be
administered by oral, parenteral (intramuscular,
intraperitoneal, intravenous (IV) or subcutaneous
30 injection), transdermal (either passively or using
iontophoresis or electroporation) or transmucosal

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1 (nasal, vaginal, rectal, or sublingual) routes of administration in dosage forms appropriate for each route of administration.

5 Solid dosage forms for oral administration include capsules, tablets, pill, powders, and granules. In such solid dosage forms, the active compound is admixed with at least one inert pharmaceutically acceptable carrier such as sucrose, lactose, or starch. Such dosage forms can also comprise, as it normal practice, additional substances other than inert diluents, e.g., lubricating, agents such as magnesium stearate. In the case of capsules, tablets and pills, the dosage forms may also comprise buffering, agents. Tablets and pills can additionally be prepared with enteric coatings.

10
15 Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, with the elixirs containing inert diluents commonly used in the art, such as water. Besides such inert diluents, compositions can also include adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring and perfuming agents.

20
25 Preparations according to this invention for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, or emulsions. Examples of non-aqueous solvents or vehicles are propylene glycol, polyethylene glycol, vegetable oils, such as olive oil and corn oil, gelatin, and injectable organic esters such as ethyl oleate. Such dosage forms 30 may also contain adjuvants such as preserving, wetting,

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1 emulsifying, and dispersing agents. They may be
sterilized by, for example, filtration through a
bacteria retaining filter, by incorporating sterilizing
agents into the compositions, by irradiating the
5 compositions, or by heating the compositions. They can
also be manufactured using sterile water, or some other
sterile injectable medium, immediately before use.

10 Compositions for rectal or vaginal
administration are preferably suppositories which may
contain, in addition to the active substance, excipients
such as cocoa butter or a suppository wax. Compositions
for nasal or sublingual administration are also prepared
with standard excipients well known in the art.

15 The dosage of active ingredient in the
compositions of this invention may be varied; however,
it is necessary that the amount of the active ingredient
shall be such that a suitable dosage form is obtained.
The selected dosage depends upon the desired therapeutic
effect, on the route of administration, and on the
20 duration of the treatment desired. Generally dosage
levels of between 0.001 to 10 mg/kg of body weight daily
are administered to mammals.

25 As can be appreciated from the disclosure
above, the present invention has a wide variety of
applications. Accordingly, the following examples are
offered by way of illustration, not by way of
limitation.

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EXAMPLE 1

SDS-PAGE gels (10-20% gradient SDS-PAGE plates, 84 x 70 x 1.0 mm, Integrated Separation Systems, Natick, MA) were stained with Coomasie Brilliant Blue R-250 (BioRad). A commercial preparation of activated difunctional polyethylene glycol (PEG-succinimidyl-propionate, SPA2, MW ca. 3400) was purchased from Shearwater Polymers, Huntsville, AL as was the monofunctional reagent, methoxy-PEG-succinimidyl-propionate, MW ca 5000. Peptide (SEQ ID NO: 8) and all other peptides were obtained from the Peptide Synthesis Facility RWJ-PRI, La Jolla, CA or Quality Controlled Biochemical, Hopkinton MA. These peptides were cyclized via oxidation of their intramolecular cysteines, amidated at the C-terminus and mass confirmed by FAB-MS. All were Ellman Reaction negative. Tris base was obtained from BioRad, Hercules, CA. (DPDPB) and trifluoracetic acid (HPLC grade) were obtained from Pierce Chemical Co., Rockford IL.

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Mono-PEG conjugation of peptide GGTYSCHFGPLTWVCKPQGG
(SEQ ID NO: 8)

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This example describes the preparation of mono-PEG conjugates of peptide (SEQ ID NO: 8), using the monofunctional amine reactive polymer analog m-SPA-PEG to be used as a control in experiments described herein. The reaction was carried out with polymer in excess (ca. 3 fold) by resuspending 142.5 mg (0.0286 mmol, MW ca. 5000) of polymer in 4 ml PBS at pH 7.5 and adding 10 mg peptide (SEQ ID NO: 8) (0.0095 mmol, MW 2092) dissolved

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1 in 1 ml of 0.1% trifluoroacetic acid. The mixture was
incubated on ice for 20 hours. The reaction was
subsequently adjusted to a final concentration 50 mM
5 Tris by the addition of 1 M tris-HCl at pH 7.5. The
reaction mixture was incubated on ice for one hour.
Analytical HPLC suggested that there were two main
reaction products of essentially equivalent magnitude
which were not baseline resolved. Preparative HPLC
10 (using the flatter gradient system described in Example
8) and conservative cuts resulted in collection of two
product peaks eluting at ca 44 and 47 minutes. After
lyophilization, 24.8 mg, 16.5 mg of each species was
recovered, respectively. Mass spectral analysis of
these two species demonstrated centroid masses of 7092
15 (peak 1) and 12036 (peak 2) indicating the coupling of
one or two PEG molecules, respectively, to the peptide
(Table I).

20 Tris inactivated polymer. Tris inactivated
polymer was formed by incubation of 5 mM SPA2 polymer
dissolved in PBS (Gibco, Gaithersburg, MD) with 50 mM
tris-HCl, pH 7.5 added and used without further
purification.

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TABLE 1
Recovery Yield of Peptide Conjugation Reaction and Apparent Molecular Mass of Product

I.D. No.	Sequence	Mass	Conjugation Reagent	Main Product Mass (centroid m/z)	Yield (% of theoretical)
8	CCTYSCHIFGPLTWVCKPQCG	2092	SPA2-PEG (MW ca. 3400)	7834	69
			m-SPA-PEG (MW ca. 5000)	7092 (peak 1) 12036 (peak 2)	-
13	GGTYSCHIFGPLTWVCKPKQ	1978	SPA2-PEG	7560	54
20	Ac-GGTYSCHIFGPLTWVCKPQCG	2133	SPA2-PEG	7862	30
14	GGLYACIDICPHITWVCPPLRG	2177	SPA2-PEG	7872	37
18	SCIIFGPLTWVCK	1175	SPA2-PEG	6326	45

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EXAMPLE 2

PEG dimerization of peptide GGTYSCHFGPLTWVCKPQGG
(SEQ ID NO: 8) (lot #1)

Examples 2 - 7 describe the dimerization of various peptides described by the present invention. The modification of peptide (SEQ ID NO: 8) was carried out by resuspending 25 mg (0.0071 mmol) of polymer in 4 ml PBS at pH 7.5, and adding a 3 fold molar excess of peptide (SEQ ID NO: 8) (0.0213 mmol, 44.5 mg, MW 2092) dissolved in 1 ml of 0.1% trifluoroacetic acid. The mixture was incubated on ice. After 3 hours of incubation, an additional 7.5 mg (0.0036 mmol) of lyophilized peptide was added, resulting in a final ratio of 3.5 moles of peptide per each mole of SPA2. The mixture was incubated an additional 17 hours on ice. The reaction mixture was adjusted to a final concentration of 50 mM Tris by the addition of 1 M tris-HCl of pH 7.5 and incubated on ice for 1 hour. The sample was subjected to analytical and preparative HPLC as described in Example 8. After preparative HPLC and lyophilization, 38 mg of PEG dimer was recovered. The theoretical yield for this experiment was 55 mg based on a calculated mass of 7600 mg/mmol for a yield of 69% (Table I).

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EXAMPLE 3

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PEG dimerization of peptide GGTYSCHFGPLTWVCKPQGG
(SEQ ID NO: 8) (lot #2)

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The modification of peptide (SEQ ID NO: 8) was carried out by resuspending 25 mg (0.0071 mmol) of polymer in 4 ml PBS at pH 7.5, and adding a ca. 3 fold molar excess of peptide (SEQ ID NO: 8) (0.0213 mmol, 45.8 mg, MW 2092) dissolved in 1 ml of 0.1% trifluoroacetic acid. The mixture was incubated on ice for 22 hours. At that time, the reaction was adjusted to a final concentration of 50 mM Tris by the addition of 1 M tris-HCl, pH 7.5. The reaction mixture was incubated on ice for 1 hour. The sample was subjected to analytical and preparative HPLC as described in Example 8. After preparative HPLC and lyophilization, 37 mg of PEG dimer was recovered. The theoretical yield for this experiment was 55 mg based on a calculated mass of 7600 mg/mmol for a yield of 68% (Table I).

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EXAMPLE 4

PEG dimerization of peptide GGTYSCHFGPLTWVCKPQ
(SEQ ID NO: 13)

The modification of peptide (SEQ ID NO: 13) was carried out by resuspending 11.2 mg (0.0033 mmol) of polymer in 2.5 ml PBS at pH 7.5, and adding a ca. 3 fold molar excess of peptide (SEQ ID NO: 13) (0.010 mmol, 20 mg, MW 1978) dissolved in 0.25 ml of 0.1% trifluoroacetic acid. The mixture was incubated on ice for 20 hours. At that time, 0.25 ml of 1 M tris-HCl at pH 7.5 was added. The reaction mixture was incubated at 4°C for one hour. The sample was subjected to analytical and preparative HPLC as described in Example 8. The main preparative reaction product peak eluted at ca 43 minutes. After preparative HPLC and lyophilization, 13.3 mg of PEG dimer was recovered. The theoretical yield for this experiment was 24.42 mg based on a calculated mass of 7400 mg/mmol for a yield of 54% (Table I).

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1 EXAMPLE 5

5 PEG dimerization of peptide Ac-GGTYSCHFGPLTWVCKPQGG
 (SEQ ID NO: 20)

The modification of peptide (SEQ ID NO: 20) was carried out by resuspending 10.5 mg (0.0031 mmol) of polymer in 2.5 ml PBS at pH 7.5, and adding a ca. 3 fold molar excess of peptide (SEQ ID NO: 20) (0.0094 mmol, 20 mg, MW 2133) dissolved in 0.25 ml of 0.1% trifluoroacetic acid and the mixture incubated at 4°C for 28 hours. At that time, the reaction as monitored by HPLC was estimated to be approximately 30% complete, the temperature was shifted to ambient and an additional 27 hour incubation provided no net increase in product. Because of possible hydrolysis of the reactive polymer, an additional 5 mg of polymer was added and the incubation was continued for an additional 16 hours. At that time, 0.25 ml of 1 M tris-HCl, pH 7.5 was added and the reaction mix was incubated at 4°C for an additional 1 hour. The sample was subjected to analytical and preparative HPLC using a flatter gradient system as described in Example 8. The main preparative reaction product peak eluting at ca 48 minutes. After preparative HPLC and lyophilization, 10.4 mg of PEG dimer was recovered. The theoretical yield for this experiment was 34.4 mg based on a calculated mass of 7650 mg/mmol for a yield of 30% (Table I).

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EXAMPLE 6

PEG dimerization of peptide (SEQ ID NO: 14)

5 The modification of peptide (SEQ ID NO: 14) was carried out by resuspending 2.6 mg (0.00076 mmol) of polymer in 3.0 ml PBS at pH 7.5 and adding a ca. 3 fold molar excess of peptide (SEQ ID NO: 14) (0.00229 mmol, 5 mg, MW 2177) dissolved in 0.1 ml of 0.1% trifluoroacetic acid. The mixture was incubated on ice for 26 hours.

10 At that time, 0.25 ml of 1 M-tris-HCl at pH 7.5 was added. The reaction mixture was incubated at 4°C for 1 hour. The sample was subjected to analytical and preparative HPLC using the flatter gradient system

15 described in Example 8. The main preparative reaction product peak eluted at ca 46 minutes. After preparative HPLC and lyophilization, 2.2 mg of PEG dimer was recovered. The theoretical yield for this experiment was 24.42 mg based on a calculated mass of 7400 mg/mmol for a yield of 37% (Table I).

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EXAMPLE 7

PEG dimerization of peptide (SEQ ID NO: 18)

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The modification of peptide (SEQ ID NO: 18) was carried out by resuspending 1.2 mg (0.00036 mmol) of polymer in 0.5 ml PBS at pH 7.5, and adding a ca. 3 fold molar excess of the peptide (0.0011 mmol, 1.5 mg, MW 2177) dissolved in 0.05 ml of 0.1% trifluoroacetic acid. 10 The mixture was incubated on ice for 20 hours. At that time, 0.1 ml of 1 M tris-HCl at pH 7.5 was added. The reaction mixture was incubated at 4°C for 1 hour. The sample was subjected to purification using an analytical HPLC system as described in Example 8. The main 15 reaction product peak eluted at ca 38 minutes. After preparative HPLC and lyophilization, 1 mg of PEG dimer was recovered. The theoretical yield for this experiment was 2.2 mg based on a calculated mass of 6150 mg/mmol for a yield of 45% (Table I).

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EXAMPLE 8

Analytical and Preparative HPLC analysis

5 The accumulation of the dimers described above
in Examples 1-7 was monitored by analytical reverse
phase HPLC. The analysis was carried out using a Vydac
C-18 Protein-Peptide column (0.46 x 25 cm, part no.
10 218TP54) and a Rainin Gradient HPLC system fitted with a
Dynamax dual wavelength detector. At injection, the
column was equilibrated in 0.1% TFA in dH₂O and was
developed with a 45 minute linear gradient (0-100%) of
15 acetonitrile (ACN) containing 0.1% TFA beginning at 10
minutes after injection. The flow rate was held
constant at 1 ml/min. Under these analytical
conditions, the SPA2 polymer and tris inactivated
polymer did not appear to bind the column while a major
reaction product with a retention time (37 minutes) was
identified (Figure 1). Peptide (SEQ ID NO: 8)
20 demonstrated a retention time of 35 minutes and the
excess peptide utilized in the reaction was clearly
distinguished from the nascent reaction products.

25 The main product reaction product peak was
purified by preparative reverse phase HPLC on the same
chromatographic system using a Vydac C-18 Protein-
Peptide column (2.2 x 25 cm, part no. 218TP15022).
Injection of the reaction mix (6 ml) occurred with the
column equilibrated at 80:20, H₂O:ACN (both containing
0.1% TFA) at a constant flow rate of 8 ml/min. After a
30 20 minute wash, the column was developed by application
of a linear gradient of 100% ACN/0/1% TFA over 60

-35-

1 minutes. The major product peak eluting at 48 minutes
was collected and lyophilized (Figure 2). These elution
conditions were subsequently modified to improve the
5 resolution of some conjugation products peptide (SEQ ID
NO: 20), mPEG-peptide (SEQ ID NO: 8), peptide (SEQ ID
NO: 14) from reaction by products. This was
accomplished by application of a flatter linear gradient
10 of 20-80% B over 60 minutes. The variation in retention
time due to different peptides and elution condition is
described as part of each synthesis example. The
materials recovered from the main product peak from each
reaction were subsequently analyzed by analytical
reverse phase HPLC, MALDI-TOF mass spectrometry, EPO
15 competitive binding potential and for in vitro
bioactivity.

The activated PEG used in these experiments
has an approximate molecular weight of 3400 and has
amine reactive succinimidyl groups on either end of the
difunctional linear polymer. This reactivity was
20 employed to couple two equivalents of peptide (SEQ ID
NO: 8) (MW= 2092) to the polymer with the concomitant
liberation of two succinimidyl moieties resulting in a
dimeric product as shown in Scheme I. Peptide (SEQ ID
NO: 8) contains two potentially reactive amines, one at
25 the N-terminus of the peptide and one in the side chain
of the single lysine within the peptide sequence, so
that a number of different connectivities between the
two molecules was possible.

30 MALDI-TOF mass spectral analysis was
supportive of the presence of the expected dimeric
product (Figure 3) as indicated by a predominant species

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1 with a centroid mass of 7661. This data shows that the
dimeric product described in the present invention was
produced using the methods described herein.

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EXAMPLE 9

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This example demonstrates the interaction of peptide (SEQ ID NO: 8), peptide (SEQ ID NO: 16), peptide (SEQ ID NO: 18) and peptide (SEQ ID NO: 13) with EPO binding protein (EBP) using a bifunctional sulphhydryl reactive crosslinker, (1,4-Di-[3'-(2'-pyridyldithio)propionamido]butane DPDPB.

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To explore the interaction of peptide (SEQ ID NO: 8) with EBP, a bifunctional sulphhydryl reactive crosslinker (DPDPB) was used in an attempt to stabilize a mimetic dependent dimeric structure. Control experiments demonstrated that the crosslinker does not inactivate the EPO binding potential of EBP or the proliferative properties of peptide (SEQ ID NO: 8). As shown in Figure 4, a dimeric EBP product was formed by co-incubation of the peptide, peptide (SEQ ID NO: 8), DPDPB and EBP. This data shows the ability of the peptide (SEQ ID NO: 8) to mediate formation of a soluble receptor dimer. To further explore this question, peptides (SEQ ID NO: 13), (SEQ ID NO: 16) and (SEQ ID NO: 18) were examined for their ability to mediate dimerization. As shown in Figure 4, lanes 7A and 8A, when peptide (SEQ ID NO: 13) was truncated at the carboxyl terminus, it retained good in vitro bioactivity and improved in vivo bioactivity, resulting in a crosslinking signal similar to peptide (SEQ ID NO: 8). However, peptide (SEQ ID NO: 18) did not appear to stabilize the dimerization signal (Figure 4, lanes 9A

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1 and 10A) whereas peptide (SEQ ID NO: 16) (Figure 4,
lanes 5A and 6A) gave a strong dimerization band. These
two peptides differ by a single N-terminal tyrosine
residue and display a similar profile in the in vitro
5 proliferation assay with peptide (SEQ ID NO: 18) being
inactive. Peptide (SEQ ID NO: 16) has an ED₅₀ of 3 μM
on murine receptor cells. Both peptides have similar
IC₅₀ values indicating that they both retain binding
activity. These results demonstrate that EBP
10 dimerization is a property of the EPO peptide series and
that the presence of the tyrosine is critical for this
activity and that this corresponds to in vitro
bioactivity.

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EXAMPLE 10

5 IMMOBILIZED EBP BASED [¹²⁵I]EPO COMPETITION BINDING
ASSAY

10 This study examined the binding capacity of
the EPO PEG dimers to bind EPO receptors.

15 The extracellular domain of the human
erythropoietin receptor (EPO binding protein, EBP) was
expressed and overproduced in E. coli. As with many
other recombinant eukaryotic proteins produced in E.
coli, the protein appeared as an insoluble product in
laboratory scale fermentations and was refolded and
purified to obtain active protein. EPO binding protein
20 produced by this method contains one free sulfhydryl
group which can be modified without effecting the
solution phase binding of ligand. In order to
immobilize the EPO binding protein for equilibrium
binding analysis and for competition binding assay, the
25 EPO binding protein was covalently attached to agarose
beads.

30 The iodoacetyl activation chemistry of
Sufolink beads (Pierce Chemical Co, Rockford, IL) is
specific for free thiols and assures that the linkage is
not easily reversible. EBP-Sufolink beads were made as
follows: Sufolink gel suspension (10 ml) was mixed with
of coupling buffer (40 ml: 50 mM Tris, pH 8.3, 5 mM
EDTA) and the gel was allowed to settle. The
supernatant was removed and the EPO binding protein
(0.3-1 mg/ml in coupling buffer) to be bound was added
directly to the washed beads. The mixture was rocked

-40-

1 gently for 30 minutes at room temperature and the beads
were allowed to settle for 1 hour at room temperature.
The supernatant was removed and retained. The beads
5 were washed twice with 20 ml of coupling buffer. The
washes were recovered as well. The beads were then
treated with 20 ml of 0.05 M cysteine for 30 minutes at
room temperature to block unbound sites. Finally, the
beads were washed with 50 ml of 1 M NaCl, then with 30
10 ml of PBS, and resuspended in 20 ml of PBS and stored at
4°C. The amount of EBP which was covalently bound to
the beads was determined by comparing the OD₂₈₀ of the
original EBP solution to the total OD₂₈₀ recovered in
the reaction supernatant and the two 20 ml washes.
Typically, 40-60% of the applied EBP remains associated
15 with the beads.

Binding assays were initiated by the addition
of EPO binding protein beads (50 µl) to individual
reaction tubes. Total binding was measured in tubes
containing 0.3-30 nM [¹²⁵I]EPO (NEN Research Products,
20 Boston MA, 100 µCi/µg). For determination of
non-specific binding, unlabelled EPO was added at a
level of 1000 fold in excess of the corresponding
[¹²⁵I]EPO concentration. Each reaction volume was
brought to 500 µl with binding buffer (PBS/0.2% BSA).
The tubes were incubated for five hours (a time period
25 experimentally determined as adequate for the
establishment of equilibrium) at room temperature with
gentle rocking. After five hours, each reaction mixture
was passed through a 1 ml pipet tip plugged with glass
wool. The tubes were washed with 1 ml wash buffer (PBS/
30 5% BSA) and this volume as well as 2 additional 1 ml

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washes were passed through the pipet tip and collected for determination of the free EPO concentration. Equilibrium binding analysis of the specific association of [¹²⁵I]EPO with EPO mimetic binding proteins immobilized on these agarose beads indicates a Kd of 5 nM ± 2 based on a linear transformation (Scatchard) of the binding isotherm (Figure 5).

Competitive binding analysis assays of candidate peptides and dimer peptides were performed as outlined below. Individual peptides were dissolved in DMSO to prepare a stock solution 1 mM. Dimer peptides were contained within PBS at a concentration of 5 mM. All reaction tubes (in duplicate) contained 50 µL of EBP beads, 0.5 nM [¹²⁵I]EPO and 0-500 µM peptide in a total of 500 µL binding buffer.

The final concentration of DMSO was adjusted to 2.5% in all peptide assay tubes. At this concentration DMSO has no detectable effect since an examination of the sensitivity of the assay to DMSO demonstrated that concentrations of up to 25% DMSO (v/v) had no deleterious effect on binding. Non-specific binding was measured in each individual assay by inclusion of tubes containing a large excess of unlabelled EPO (1000 nM). Initial assay points with no added peptide were included in each assay to determine total binding. Binding mixtures were incubated overnight at room temperature with gentle rocking. The beads were then collected using Micro-columns (Isolab, Inc.) and washed with 3 mL of wash buffer. The columns containing the washed beads were placed in 12 x 75 mm glass tubes and bound radioactivity levels determined in

-42-

1 a gamma counter. The amount of bound [125 I]EPO was
expressed as a percentage of the control (total=100%)
binding and plotted versus the peptide concentration
after correction for non-specific binding. The IC₅₀ was
5 defined as the concentration of the analyte which
reduced the binding of [125 I]EPO to the EBP beads by
50%. All data are reported as relative to peptide (SEQ
ID NO: 8) which demonstrated an IC₅₀ of 5 μ M.

10 Competitive binding analysis revealed an IC₅₀
of 20 μ M for the purified dimer, a value four fold
greater than peptide (SEQ ID NO: 8) in the same assay
(Figure 5 and Table II). Polymer alone, which was
15 inactivated by treatment with Tris-HCl, demonstrated a
detectable competition binding signal but this signal
was modest (<10%) at the IC₅₀ of the PEG-peptide (SEQ ID
NO: 8) dimer.

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TABLE II

Table II. Binding and Cell Proliferation Studies

id	Relative Binding*	EPO-ED ₅₀ (μM)*	
		enzymic receptor	transduced human receptor
D. No. 8	1	0.1	0.09
zis inact. polymer	60	1A ²	1A
q. I.D. No. 8 covalent dimer #1	4	0.01 (10X)	0.0015 (60X)
q. I.D. No. 8 covalent dimer #2	3	0.01 (10X)	0.002 (45X)
D. No. 13	1.6	0.08	0.02
q. I.D. No. 13 covalent dimer	3	0.01 (8X)	0.002 (10X)
D. No. 20 (N-acetyl)	4	0.03	0.06
q. I.D. No. 20 covalent dimer	12	0.2 (-7X)	0.05
D. No. 14 (terminal NH ₂)	0.6	0.1	0.08
q. I.D. No. 14 covalent dimer	-	0.006 (16X)	0.001 (80X)

* Assayed reagents to achieve one half maximal level of EPO-dependent proliferation (11 pm²)
 Binding relative to Seq. I.D. No. 8
²Ac-inactive
 Note that all peptides are cyclic and were synthesized as COOH-terminal amides (-CONH₂)

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EXAMPLE 11

EPO DEPENDENT CELL PROLIFERATION ASSAYS

5 This example shows the improved potency of PEG-EPO peptide dimers to EPO receptors in human and murine cell lines.

10 Cell line FDC-P1/ER, an EPO-dependent line expressing the murine EPO receptor, was grown and maintained as described previously (Carroll et al. 1991). Also employed was cell line FDC-P1/trER expressing a functional truncated human EPO receptor (missing the C-terminal 40 amino acids). Both cell lines exhibit EPO dependent cellular proliferation.

15 Briefly, cells were maintained in RPMI 1640 media (Gibco/BRL) containing 10% heat-inactivated fetal calf serum and 10 units/ml of recombinant human EPO. For the cellular proliferation assay, FDC-P1/ER or FDC-P1/trER cells were grown to stationary phase, centrifuged, washed with RPMI 1640 media (no EPO), and plated in EPO minus media for 24 hr.

20 After 24 hours, the cells were counted, resuspended at 800,000 cells/ml and dispensed at 40,000 cells/well. Stock solutions of the peptide dimer (5 mM in PBS) and peptide (10 mM in DMSO) were prepared and dispensed in triplicate to final concentrations of 1 x 10⁻¹⁰ M through 1 x 10⁻³ M and adjusted to a final volume of 0.2 ml. Final DMSO concentrations of 0.1% (V/V, maximal) or less were found to have no cellular toxicity or stimulatory effects. A standard EPO dose response curve was generated with each assay series.

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1 After a 42 hr incubation at 37°C (ca. 2 cell doublings)
1μCi/well of [³H] thymidine was added and the incubation
continued for 6 hr at which time the cells were
harvested and counted to assess [³H]thymidine
5 incorporation as a measure of cell proliferation.
Results are expressed as the amount of peptide or dimer
peptide necessary to yield one half of the maximal
activity obtained with recombinant EPO.

10 As shown in Figure 5 and Table II, the initial
lot of PEG-peptide (SEQ ID NO: 8) dimer demonstrated
ED₅₀ values of 0.01 μm and 0.0015 μm in EPO responsive
cell lines containing the murine or human EPO receptor,
respectively. In both cell lines, the parent peptide,
15 peptide (SEQ ID NO: 8), demonstrated an ED₅₀ of 0.1 μM,
indicating an increase in potency of 10 fold in the
murine receptor line and almost 60 fold in the human
receptor containing cells. Thus, the dimer was clearly
more potent in murine and human lines than the peptides
themselves. This was confirmed by generation of a
20 second synthesis lot of PEG-peptide (SEQ ID NO: 8) dimer
which resulted in a 10 and 45 fold increase in potency
in the murine and human lines, respectively. Polymer
alone, which was inactivated by treatment with Tris-HCl,
25 demonstrated no activity in the cell proliferation
assay.

30 A second EPO mimetic peptide, peptide (SEQ ID
NO: 13), with the sequence GGTYSCHFGPLTWVCKPQ, was also
subjected to a similar PEG dimerization protocol as that
described above for peptide (SEQ ID NO: 8). The dimer
product of PEG-peptide (SEQ ID NO: 13) is also more
active than the unconjugated parent compound (Table II).

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1 Both of these dimer peptides have ultimate ED₅₀ values
near 0.002μM. In spite of this more modest increase,
the experimental evidence clearly indicates that the
dimerization of these peptides with PEG results in
5 improved potency.

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1 EXAMPLE 12

5 To further examine the connectivity of the peptides of the present invention to PEG, peptide molecules, which contained only an internal lysine group were used peptide (SEQ ID NO: 8) analog acetylated at the N-terminus peptide (SEQ ID NO: 20) and a sequence analog peptide (SEQ ID NO: 14) which only had a reactive N-terminal amine were PEG dimerized. In vitro proliferation data of these compounds suggest that potential dimerization through the free amino terminus has the most profound effect on bioactivity giving rise to a species about 80 fold more active than the monomeric parent peptide (SEQ ID NO: 14) dimer.

10 Conjugation through the lysine side chain had no real effect on activity peptide (SEQ ID NO: 20) as did mono-PEG or di-PEG conjugation (Table III). This data indicates that the creation of a head to head dimer (both peptides attached through the N-terminus) using a PEG linker greatly enhances the potency of EPO peptides and approaches a level almost two logs greater than the free parent peptide. Further, this effect was not observed upon simple covalent attachment of linear PEG to peptide (SEQ ID NO: 8) indicating that dimerization is a critical determinant for this increased activity.

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TABLE III

Table III. Binding and Cell Proliferation Studies mPEG/

Compound	Relative Binding*	EPO-ED ₅₀ (μM)*	
		mouse receptor	truncated human receptor
Seq. I.D. No. 8	1	0.1	0.09
mPEG/Seq. I.D. No. 8, peak #1	~60	2	0.1
mPEG/Seq. I.D. No. 8, peak #2	>40	1	0.4

* Amount required to achieve the half maximal level of EPO dependent proliferation (11 pM)

1 ND=Not determined

2 Absent

*Binding relative = Seq. I.D. No. 8

Note that all peptides are cyclic and were analyzed as COOH terminus amide (-CONH₂)

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EXAMPLE 13

Polycythemic Exhypoxic Mouse Bioassay.

5 This study demonstrates the ability of peptide
 (SEQ ID NO: 8)/PEG-dimers to retain in vivo bioactivity.
Peptides were assayed for in vivo activity in the
polycythemic mouse bioassay adapted from the method
described by Cotes and Bangham (1961), Nature 191: 1065-
10 1067. BDF1 mice were allowed to acclimate to ambient
conditions for 7-10 days. Body weights were determined
for all animals. Low weight animals (<15 grams) were
not used. Mice were introduced to hypobaric chambers
15 with a 24 hour conditioning cycle consisting of 0.40%
+/- 0.02 atm. for 18 hours followed by 6 hours at
ambient pressure for a total of 14 days. Following the
14 day period, mice were placed in ambient pressure for
72 hours prior to dosing. Test samples or recombinant
20 Human Erythropoietin (rHuEPO) standards were diluted in
an assay vehicle consisting of Phosphate Buffered Saline
(PBS)-0.1% Bovine Serum Albumin (BSA). Peptide sample
stock solutions (excluding peptide dimers) were first
solubilized in dimethyl sulfoxide (DMSO). Control
groups included one group of vehicle alone, and one
25 group of (DMSO) at final concentration of 1%.

Each dose group contained 10 mice. Mice were
30 injected subcutaneously (scruff of neck) with 0.5 ml of
the appropriate sample. Forty eight hours following the
sample injection, the mice were administered an
intraperitoneal injection of 0.2 ml of [⁵⁵Fe]

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(approximately 18.0 milliCuries/milligram, Dupont, NEN) and 0.75 microCuries/Mouse.

Mouse body weights were determined twenty four hours following [⁵⁹Fe] administration and the mice were sacrificed forty eight hours following the [⁵⁹Fe] injection. Blood was collected from each animal by cardiac puncture and hematocrits were determined (heparin was used as the anticoagulant). Each blood sample (0.2 ml) was analyzed for [⁵⁹Fe] incorporation using a Packard gamma counter. Non-responder mice (i.e., those mice with radioactive incorporation less than the negative control group) were eliminated from the appropriate data set. Mice that had hematocrit values less than 53% were also eliminated.

This assay examined the ability of an exogenously administered compound to induce new red blood cell synthesis, or in other words to function as EPO or an EPO mimetic. The results are derived from sets of 10 animals for each experimental dose. As shown in Figure 7 and Table IV, the data suggests that on a mole equivalent basis, peptide (SEQ ID NO: 8)/PEG-dimer is about 10 fold more active than peptide (SEQ ID NO: 8) monomer. These results are consistent with in vitro results in which increased potency values of 10 fold was observed on murine EPO-R bearing cells.

TABLE IV

Table 4. Exotoxic Mouse Bioassay Study of PEG Dimer Activity

Compound	Amount required for equivalence to 0.025U EPO (nmol) n=10
Seq. I.D. No. 8	3.8

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EXAMPLE 14

This example shows that an inactive truncation analog of peptide (SEQ ID NO: 8), which lacks the critical tyrosine peptide (SEQ ID NO: 18), (SCHFGPLTWVCK), can be converted to an agonist on the human EPO receptor cell line by PEG dimerization. In this experiment, a 10^{-5} M concentration of the parent peptide had no activity above background while the dimeric peptide exhibited a level of proliferation twice as many cpm as background. As shown in Figure 8, the peptide alone (open squares) did not induce proliferation of the EPO responsive cells but upon PEG dimerization (open diamonds) a significant agonist effect was observed. Approximately twice as many cpm incorporated over non-stimulated cells at 10^{-5} M added peptide dimer. The replicate error bars represent the standard deviation of three assay points per concentration of peptide or peptide dimer.

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1 Exhypoxic polycvthemic mouse bioassay:

PEG dimer and the monomer parent peptide RWJ 61718 were compared in the exhypoxic mouse bioassay (Table 1). This peptide exhibited an 80 fold increase in in vitro activity upon dimerization. Murine studies (in vivo) of the activity of the dimer compared to the monomer peptide revealed a 250 fold increase in activity of RWJ 61718 upon dimerization. Cellular proliferation studies on this dimer peptide in murine receptor containing cells demonstrated a 16 fold increase over the monomer indicating that the 250 fold increase in vivo might be attributable to other factors such as altered metabolism or prolonged circulatory half-life which occur upon PEG dimerization of the peptide sequence. Thus, in addition to the effect of dimerization alone, the PEG modification has an effect which impacts in vivo activity and may be specific to individual peptide sequences.

15 Cell associated EPO receptor competition binding assay.

A competitive binding analysis of the ability of selected monomer peptides and their cognate dimer products to compete with radiolabelled EPO binding for cell associated human EPO receptors was performed Erythropoietin Receptor Competition Binding Analysis was preformed as follows. TF-1 cells were maintained in RPMI 1640, 10% fetal calf serum, 1% L-glutamine, 1% penicillin, 0.1% streptomycin and 1 ng/ml of GM-CSF. [125]-EPO was obtained from NEN Research Products. Cells were centrifuged and washed 1 x with binding buffer (RPMI 1640, 5% BSA, 25 mM Hepes, pH 7.5, 0.02% sodium azide) resuspended in binding buffer, and counted using trypan blue as an indicator of viability. Each reaction contained approximately 5×10^5 cells, [125]-EPO (0.5 nM), no competitor or peptide or dimer preparation in a final volume of 200 μ l. The binding reactions (in duplicate) were incubated overnight at 4°C. Following binding, the tubes were centrifuged at 12,000 rpm for 1 min at 4°C in a

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1 refrigerated centrifuge. The supernatant was removed, the cell
pellet resuspended in 100 µl of binding buffer, and the cell
suspension layered onto 0.7 ml of bovine calf serum. The tubes were
5 centrifuged at 12,000 rpm for 5 min at 4°C, the supernatant was
removed, the bottom of the tubes snipped off, and the cell pellets
counted in a Micromedic ME plus gamma counter. Non-specific binding
was determined by incubating cells with [¹²⁵I]-EPO and a 100-fold
excess of non-radioactive EPO. These data demonstrate increases in
apparent binding competitive affinity of 3.0 fold, 3.2 fold and 80
10 fold for peptides RWJ 61233, RWJ 61596 and RWJ 61718, respectively
(Table 2). In vivo proliferation studies with these peptides and
their dimer derivatives reveal increases in potency of ea. 50 fold,
10 fold and 80 fold, respectively, indicating that the magnitude of
increased binding affinity is exceeded by the functional potency of
the peptide for two of the three species. Thus, the effect of
15 dimerization and subsequent increase in activity may be one in which
the efficiency of receptor stimulation is improved by limiting the
lateral diffusion of the receptors away from a binding event.
Peptide dimerization therefore likely results in entropic rather
than enthalpic gains upon mimetic ligand-receptor association for
some peptide dimer sequences.

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Unlike the EBP-bead EPO competitive binding assay where peptide
dimerization negatively impacted the ability of PEG dimer peptides
to compete for receptor binding, the ability to compete for cell
associated receptors is increased by dimerization. This may be due
25 to the ability of the cell associated receptor to dimerize while the
immobilized EBP monomer likely cannot.

30 Conversion of inactive to active peptide RWJ 61177 was further
studied. An improved and expanded study was performed which
confirmed our earlier observation of conversion to an active peptide
(Figure 6, Panel D).

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TABLE IV. EXHYPOXIC MOUSE BIOASSAY STUDY OF PEG DIMER ACTIVITY

Compound	Amount required for equivalency to 0.025 U EPO (nmol) n=10
RWJ 61233 (seq. ID #8)	3.8
SAP2/61233, covalent dimer	0.28
RWJ 61718 (seq. ID #14)	18
SAP2/61718, covalent dimer	0.07

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TABLE V. EPO COMPETITIVE BINDING ANALYSIS OF CELL ASSOCIATED RECEPTORS

Compound	IC ₅₀ (μ M)
RWJ 61233 (seq ID #8)	3.8
SAP2/61233, covalent dimer	0.28
RWJ 61718 (seq ID#14)	18
SAP2/61718, covalent dimer	0.07

SEQUENCE LISTING

GENERAL INFORMATION:

i) APPLICANT: Johnson, Dana L
Zivin, Robert A

ii) TITLE OF INVENTION: AGONIST PEPTIDE DIMERS

iii) NUMBER OF SEQUENCES: 93

iv) CORRESPONDENCE ADDRESS:

- (A) ADDRESSEE: Frank S. DiGiglio
- (B) STREET: 400 Garden City Plaza
- (C) CITY: Garden City
- (D) STATE: New York
- (E) COUNTRY: U.S.A..
- (F) ZIP: 11530

v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER: US 08/484,135
- (B) FILING DATE: 07-JUN-1995
- (C) CLASSIFICATION:

ii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: DiGiglio, Frank S
- (B) REGISTRATION NUMBER: 31,346
- (C) REFERENCE/DOCKET NUMBER: 9594

ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: (516) 742-4343
- (B) TELEFAX: (516) 742-4366

INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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(c) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION: 1..10
- (D) OTHER INFORMATION: /note= "Xaa(Pos1) can be C,A, α -amino- γ -bromobutyric acid or Hoc; Xaa(Pos2) can be R,H,L or W; Xaa(Pos3) can be M,F or I; Xaa(Pos6) can be any one of the 20 L-amino acids or the stereoisomeric D-amino acids; Xaa(Pos9) can be D,E,I,L or V; and Xaa(Pos10) can be C,A, α -amino- γ -bromobutyric acid or Hoc, provided that either Xaa(Pos1) or Xaa(Pos10) is C or Hoc"

(i) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Xaa Xaa Xaa Gly Pro Xaa Thr Trp Xaa Xaa
5 10

INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION: 1..12
- (D) OTHER INFORMATION: /note= "Xaa(Pos2) and Xaa(Pos8) can be any one of the 20 L-amino acids; Xaa(Pos3) can be C,A, α -amino- γ -bromobutyric acid or Hoc; Xaa(Pos4) can be R,H,L or W; Xaa(Pos5) can be M,F or I; Xaa(Pos11) can be D,E,I,L or V; and Xaa(Pos12) can be C,A, α -amino- γ -bromobutyric acid or Hoc, provided that either Xaa(Pos3) or Xaa(Pos12) is C or Hoc"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Tyr Xaa Xaa Xaa Xaa Gly Pro Xaa Thr Trp Xaa Xaa
1 5 10

INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 16 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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:> FEATURE:

- (A) NAME/KEY: Peptide
(B) LOCATION: 1..16
(D) OTHER INFORMATION: /note= "Xaa(Pos1), Xaa(Pos 3), Xaa(Pos9),
Xaa(Pos14), Xaa(Pos15) and Xaa(Pos16) can be any one of 20
L-amino-acids; Xaa(Pos4) can be C,A, α -amino- γ -bromobutyric acid
or Hoc; Xaa(Pos5) can be R,H,L or W; Xaa(Pos6) can be M,F or I;
Xaa(Pos12) can be D,E,I,L or V; and Xaa(Pos13) can be C,A, α -
amino- γ -bromobutyric acid or Hoc, provided that either Xaa(Pos4)
or Xaa(Pos13) is C or Hoc"

1) SEQUENCE DESCRIPTION: SEQ ID NO:3:

aa Tyr Xaa Xaa Xaa Gly Pro Xaa Thr Trp Xaa Xaa Xaa Xaa Xaa
 5 10 15

INFORMATION FOR SEQ ID NO:4:

i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Xaa Tyr Xaa Cys Xaa Xaa Gly Pro Xaa Thr Trp Xaa Cys Xaa Xaa Xaa
 1 5 10 15

NEFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Peptide
(B) LOCATION: 1..16
(D) OTHER INFORMATION: /note= "Xaa(Pos1), Xaa(Pos3) and Xaa(Pos16)
can be any one of the 20 L-amino acids; Xaa(Pos5) can be R or H;
Xaa(Pos6) can be F or M; Xaa(Pos9) can be I,L,T,M or V;
Xaa(Pos12) can be D or V; Xaa(Pos14) can be C,W,L,Q,R,S or T;
Xaa(Pos15) can be A,G,P,R or Y"

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i) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Xaa Tyr Xaa Cys Xaa Xaa Gly Pro Xaa Thr Trp Xaa Cys Xaa Xaa Xaa
1 5 10 15

FORMATION FOR SEQ ID NO:6:

i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION: 1..16
- (D) OTHER INFORMATION: /note= "Xaa(Pos1) can be D,E,L,N,S,T or V; Xaa(Pos3) can be A,H,K,L,M,S or T; Xaa(Pos5) can be R or H; Xaa(Pos6) can be F or M; Xaa(Pos9) can be I,L,T,M or V; Xaa(Pos12) can be D or V; Xaa(Pos14) can be K,R,S or T; Xaa(Pos15) is P and Xaa(Pos16) can be any one of the 20 L-amino acids"

xii) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Xaa Tyr Xaa Cys Xaa Xaa Gly Pro Xaa Thr Trp Xaa Cys Xaa Xaa Xaa
1 5 10 15

NFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Gly Gly Leu Tyr Leu Cys Arg Phe Gly Pro Val Thr Trp Asp Cys Gly
1 5 10 15

Tyr Lys Gly Gly
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FORMATION FOR SEQ ID NO:8:

- i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

iii) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ly Gly Thr Tyr Ser Cys His Phe Gly Pro Leu Thr Trp Val Cys Lys
5 10 15

ro Gln Gly Gly
20

FORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Gly Gly Asp Tyr His Cys Arg Met Gly Pro Leu Thr Trp Val Cys Lys
1 5 10 15

Pro Leu Gly Gly
20

FORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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(i) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Val Gly Asn Tyr Met Cys His Phe Gly Pro Ile Thr Trp Val Cys Arg
1 5 10 15

Pro Gly Gly
20

INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Gly Gly Val Tyr Ala Cys Arg Met Gly Pro Ile Thr Trp Val Cys Ser
1 5 10 15

Pro Leu Gly Gly
- 20

INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Val Gly Asn Tyr Met Ala His Met Gly Pro Ile Thr Trp Val Cys Arg
1 5 10 15

Pro Gly Gly

INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 amino acids
- (B) TYPE: amino acid

- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

i) MOLECULE TYPE: peptide

ii) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Iy Gly Thr Tyr Ser Cys His Phe Gly Pro Leu Thr Trp Val Cys Lys
5 10 15

ro Gln

FORMATION FOR SEQ ID NO:14:

- i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Gly Gly Leu Tyr Ala Cys His Met Gly Pro Met Thr Trp Val Cys Gln
1 5 10 15

Pro Leu Arg Gly
20

INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Thr Ile Ala Gln Tyr Ile Cys Tyr Met Gly Pro Glu Thr Trp Glu Cys
1 5 10 15

Arg Pro Ser Pro Lys Ala
20

INFORMATION FOR SEQ ID NO:16:

- i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 13 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- i) MOLECULE TYPE: peptide

- i) SEQUENCE DESCRIPTION: SEQ ID NO:16:

hr Ser Cys His Phe Gly Pro Leu Thr Trp Val Cys Lys
5 10

INFORMATION FOR SEQ ID NO:17:

- i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 11 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- ii) MOLECULE TYPE: peptide

- xii) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Tyr Cys His Phe Gly Pro Leu Thr Trp Val Cys
1 5 10

INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 12 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: peptide

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Ser Cys His Phe Gly Pro Leu Thr Trp Val Cys Lys
1 5 10

INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 12 amino acids

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- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

i) MOLECULE TYPE: peptide

c) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION: 1..12
- (D) OTHER INFORMATION: /note= "Xaa(Pos1) can be any one of the 20 L-amino acids; except that Xaa(Pos1) may or may not be Y and Xaa(Pos1) may be any non-naturally occurring aromatic acid analog when Xaa(Pos1) is Y. Xaa(Pos2) and Xaa(Pos8) can be any one of the 20 L-amino acids; Xaa(Pos3) can be C,A, α -amino- γ -bromobutyric acid or Hoc; Xaa(Pos4) can be R,H,L or W; Xaa(Pos5) can be M,F or I; Xaa(Pos11) can be D,E,I,L or V and Xaa(Pos12) can be C,A, α -amino- γ -bromobutyric acid or Hoc provided that either Xaa(Pos3) or Xaa(Pos12) is C or Hoc"

:i) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Xaa Xaa Xaa Xaa Xaa Gly Pro Xaa Thr Trp Xaa Xaa
5 10

INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xii) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Gly Gly Thr Tyr Ser Cys His Phe Gly Pro Leu Thr Trp Val Cys Lys
1 5 10 15

Pro Gln Gly Gly
20

INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

i) MOLECULE TYPE: peptide

ii) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Gly Thr Tyr Arg Cys Ser Met Gly Pro Met Thr Trp Val Cys Leu
5 10 15
Pro Met Gly Gly
20

FORMATION FOR SEQ ID NO:22:

i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xii) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Gly Gly Met Tyr Ser Cys Arg Met Gly Pro Met Thr Trp Val Cys Gly
1 5 10 15
Pro Ser Gly Gly
20

FORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Gly Gly Trp Ala Trp Cys Arg Met Gly Pro Ile Thr Trp Val Cys Ser
1 5 10 15
Ala His Gly Gly
20

FORMATION FOR SEQ ID NO:24:

- i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- .ii) MOLECULE TYPE: peptide

- .(i) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Gly Gly Met Tyr Ser Cys Arg Met Gly Pro Met Thr Trp Val Cys Ile
1 5 10 15

Pro Tyr Gly Gly
20

INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: peptide

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Gly Gly Glu Tyr Lys Cys Tyr Met Gly Pro Ile Thr Trp Val Cys Lys
1 5 10 15

Pro Glu Gly Gly
20

INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: peptide

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i) SEQUENCE DESCRIPTION: SEQ ID NO:26:

ly Gly Asp Tyr Thr Cys Arg Met Gly Pro Met Thr Trp Ile Cys Thr
5 10 15
.la Thr Gly Gly
20

INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xii) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Gly Gly Asn Tyr Leu Cys Arg Phe Gly Pro Gly Thr Trp Asp Cys Thr
1 5 10 15
Gly Phe Arg Gly
20

INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Gly Gly Asn Tyr Val Cys Arg Met Gly Pro Ile Thr Trp Ile Cys Thr
1 5 10 15
Pro Ala Gly Gly
20

INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid

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- (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

i) MOLECULE TYPE: peptide

ii) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Gly Lys Asp Val Cys Arg Met Gly Pro Ile Thr Trp Asp Cys Arg
5 10 15
Ser Thr Gly Gly
20

INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xii) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Gly Gly Ser Tyr Leu Cys Arg Met Gly Pro Thr Thr Trp Leu Cys Thr
1 5 10 15
Ala Gln Arg Gly Gly Gly Asn
20

INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Gly Gly Asn Tyr Leu Cys Arg Met Gly Pro Ala Thr Trp Val Cys Gly
1 5 10 15
Arg Met Gly Gly
20

FORMATION FOR SEQ ID NO:32:

- i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

.i) MOLECULE TYPE: peptide

:i) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Gly Gly Glu Tyr Lys Cys Arg Met Gly Pro Leu Thr Trp Val Cys Gln
1 5 10 15
Tyr Ala Gly Gly
20

INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

.ii) MOLECULE TYPE: peptide

:xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Gly Gly Asp Tyr Thr Cys Arg Met Gly Pro Met Thr Trp Ile Cys Thr
1 5 10 15
Ala Thr Arg Gly
20

INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Gly Gly Val Tyr Val Cys Arg Met Gly Pro Leu Thr Trp Glu Cys Thr
1 5 10 15

Ala Ser Gly Gly
20

INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Gly Gly Glu Tyr Ser Cys Arg Met Gly Pro Met Thr Trp Val Cys Ser
1 5 10 15

Pro Thr Gly Gly
20

INFORMATION FOR SEQ ID NO:36:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Gly Gly Glu Tyr Leu Cys Arg Met Gly Pro Ile Thr Trp Val Cys Glu
1 5 10 15

Arg Tyr Gly Gly
20

INFORMATION FOR SEQ ID NO:37:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid

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- (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

i) MOLECULE TYPE: peptide

ii) SEQUENCE DESCRIPTION: SEQ ID NO:37:

ly Gly Asn Tyr Ile Cys Arg Met Gly Pro Met Thr Trp Val Cys Thr
5 10 15
la His Gly Gly
20

FORMATION FOR SEQ ID NO:38:

- i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Gly Gly Asp Tyr Leu Cys Arg Met Gly Pro Ala Thr Trp Val Cys Gly
1 5 10 15
Arg Met Gly Gly
20

FORMATION FOR SEQ ID NO:39:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Gly Gly Leu Tyr Leu Cys Arg Phe Gly Pro Val Thr Trp Asp Cys Gly
1 5 10 15
Tyr Lys Gly Gly
20

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INFORMATION FOR SEQ ID NO:40:

- i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

iii) SEQUENCE DESCRIPTION: SEQ ID NO:40:

ly Gly Leu Tyr Ser Cys Arg Met Gly Pro Ile Thr Trp Val Cys Thr
5 10 15

Cys Ala Gly Gly
20

INFORMATION FOR SEQ ID NO:41:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Gly Gly Gly Tyr His Cys Arg Met Gly Pro Met Thr Trp Val Cys Arg
1 5 10 15

Pro Val Gly Gly
20

INFORMATION FOR SEQ ID NO:42:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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) SEQUENCE DESCRIPTION: SEQ ID NO:42:

y Gly Thr Tyr Ser Cys His Phe Gly Pro Leu Thr Trp Val Cys Lys
5 10 15
o Gln Gly Gly
20

FORMATION FOR SEQ ID NO:43:

- i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

iii) SEQUENCE DESCRIPTION: SEQ ID NO:43:

ly Gly Ile Tyr Lys Cys Leu Met Gly Pro Leu Thr Trp Val Cys Thr
5 10 15
Pro Asp Gly Gly
20

FORMATION FOR SEQ ID NO:44:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Gly Gly Leu Tyr Ser Cys Leu Met Gly Pro Ile Thr Trp Leu Cys Lys
1 5 10 15
Pro Lys Gly Gly
20

FORMATION FOR SEQ ID NO:45:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid

- (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION: SEQ ID NO:45:

Gly Asp Tyr His Cys Arg Met Gly Pro Leu Thr Trp Val Cys Lys
5 10 15

Leu Gly Gly
20

FORMATION FOR SEQ ID NO:46:

- i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

iii) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Iy Gly Asp Tyr Ser Cys Arg Met Gly Pro Thr Thr Trp Val Cys Thr
5 10 15

ro Pro Gly Gly
20

FORMATION FOR SEQ ID NO:47:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Gly Gly Asp Tyr Trp Cys Arg Met Gly Pro Ser Thr Trp Glu Cys Asn
1 5 10 15

Ala His Gly Gly
20

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FORMATION FOR SEQ ID NO:48:

i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

iii) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Gly Gly Lys Tyr Leu Cys Ser Phe Gly Pro Ile Thr Trp Val Cys Ala
1 5 10 15

Arg Tyr Gly Gly
20

INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Gly Gly Leu Tyr Lys Cys Arg Leu Gly Pro Ile Thr Trp Val Cys Ser
1 5 10 15

Pro Leu Gly Gly
20

INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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i) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Gly Ser Tyr Thr Cys Arg Phe Gly Pro Glu Thr Trp Val Cys Arg
1 5 10 15
Pro Asn Gly Gly
20

INFORMATION FOR SEQ ID NO:51:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Gly Gly Ser Tyr Ser Cys Arg Met Gly Pro Ile Thr Trp Val Cys Lys
1 5 10 15
Pro Gly Gly Gly
20

INFORMATION FOR SEQ ID NO:52:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Gly Gly Ser Tyr Thr Cys Arg Met Gly Pro Ile Thr Trp Val Cys Leu
1 5 10 15
Pro Ala Gly Gly
20

INFORMATION FOR SEQ ID NO:53:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid

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- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

i) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

Gly Gly Leu Tyr Glu Cys Arg Met Gly Pro Met Thr Trp Val Cys Arg
5 10 15

Pro Gly Gly Gly
20

INFORMATION FOR SEQ ID NO:54:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xii) SEQUENCE DESCRIPTION: SEQ ID NO:54:

Gly Gly Asp Tyr Thr Cys Arg Met Gly Pro Ile Thr Trp Ile Cys Thr
1 5 10 15

Lys Ala Gly Gly
20

INFORMATION FOR SEQ ID NO:55:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(iii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

Gly Gly Val Tyr Ser Cys Arg Met Gly Pro Thr Thr Trp Glu Cys Asn
1 5 10 15

A ? Tyr Val Gly
20

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INFORMATION FOR SEQ ID NO:56:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

Gly Gly Ala Tyr Leu Cys His Met Gly Pro Ile Thr Trp Val Cys Arg
1 5 10 15

Pro Gln Gly Gly
20

INFORMATION FOR SEQ ID NO:57:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

Gly Gly Glu Tyr Ser Cys Arg Met Gly Pro Asn Thr Trp Val Cys Lys
1 5 10 15

Pro Val Gly Gly
20

INFORMATION FOR SEQ ID NO:58:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

i) MOLECULE TYPE: peptide

ii) SEQUENCE DESCRIPTION: SEQ ID NO:61:

Gly Val Tyr Lys Cys Arg Met Gly Pro Leu Thr Trp Glu Cys Arg
5 10 15
Pro Thr Gly Gly
20

INFORMATION FOR SEQ ID NO:62:

- i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

Gly Gly Asp Tyr Asn Cys Arg Phe Gly Pro Leu Thr Trp Val Cys Lys
1 5 10 15
Pro Ser Gly Gly
20

INFORMATION FOR SEQ ID NO:63:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

Gly Gly Ser Tyr Leu Cys Arg Phe Gly Pro Thr Thr Trp Leu Cys Ser
1 5 10 15
Ser Ala Gly Gly
20

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i) SEQUENCE DESCRIPTION: SEQ ID NO:58:

Gly Leu Tyr Leu Cys Arg Met Gly Pro Val Thr Trp Glu Cys Gln
5 10 15
Arg Gly Gly
20

FORMATION FOR SEQ ID NO:59:

i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

.i) MOLECULE TYPE: peptide

:ii) SEQUENCE DESCRIPTION: SEQ ID NO:59:

Gly Gly Leu Tyr Thr Cys Pro Met Gly Pro Ile Thr Trp Val Cys Leu
1 5 10 15
Leu Pro Gly Gly
20

NFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

Gly Gly Leu Tyr Thr Cys Arg Met Gly Pro Val Thr Trp Val Cys Thr
1 5 10 15
Gly Ala Gly Gly
20

INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid

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i) SEQUENCE DESCRIPTION: SEQ ID NO:66:

ly Gly Trp Val Thr Cys Arg Met Gly Pro Ile Thr Trp Val Cys Gly
5 10 15

al His Gly Gly
20

INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

Gly Gly Gln Leu Leu Cys Gly Ile Gly Pro Ile Thr Trp Val Cys Arg
1 5 10 15

Trp Val Gly Gly
20

INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(i-) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

Gly Gly Lys Tyr Ser Cys Phe Met Gly Pro Thr Thr Trp Val Cys Ser
1 5 10 15

Pro Val Gly Arg Gly Val
20

INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid

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- (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION: SEQ ID NO:77:

Gly Leu Tyr Leu Cys Arg Met Gly Pro Gln Thr Trp Met Cys Gln
5 10 15

Gly Gly Gly
20

FORMATION FOR SEQ ID NO:78:

- i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

iii) SEQUENCE DESCRIPTION: SEQ ID NO:78:

Ala Gly Asp Tyr Val Cys Arg Met Gly Pro Met Thr Trp Val Cys Ala
5 10 15

Arg Tyr Gly Arg
20

FORMATION FOR SEQ ID NO:79:

- iv) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

v) MOLECULE TYPE: peptide

vi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

Lys Gly Gly Trp Tyr Ser Cys Leu Met Gly Pro Met Thr Trp Val Cys Lys
1 5 10 15

Ala His Arg Gly
20

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FORMATION FOR SEQ ID NO:80:

- i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

ci) SEQUENCE DESCRIPTION: SEQ ID NO:80:

Gly Gly Lys Tyr Tyr Cys Trp Met Gly Pro Met Thr Trp Val Cys Ser
1 5 10 15

Pro Ala Gly Gly
20

NFORMATION FOR SEQ ID NO:81:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

Gly Gly Tyr Val Met Cys Arg Ile Gly Pro Ile Thr Trp Val Cys Asp
1 5 10 15

Ile Pro Gly Gly
20

INFORMATION FOR SEQ ID NO:82:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

- 34 -

(i) SEQUENCE DESCRIPTION: SEQ ID NO:82:

Gly Ser Cys Leu Gln Cys Cys Ile Gly Pro Ile Thr Trp Val Cys Arg
5 10 15
Asn Ala Gly Gly
20

INFORMATION FOR SEQ ID NO:83:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

Gly Gly Asn Tyr Phe Cys Arg Met Gly Pro Ile Thr Trp Val Cys Gln
1 5 10 15
Arg Ser Val Gly
20

INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

Gly Gly Glu Tyr Ile Cys Arg Met Gly Pro Leu Thr Trp Glu Cys Lys
1 5 10 15
Arg Thr Gly Gly
20

INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid

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- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

Gly Gly Leu Tyr Ala Cys Arg Met Gly Pro Ile Thr Trp Val Cys Lys
1 5 10 15

Tyr Met Ala Gly
20

INFORMATION FOR SEQ ID NO:86:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

Gly Gly Gln Tyr Leu Cys Thr Phe Gly Pro Ile Thr Trp Leu Cys Arg
1 5 10 15

Gly Ala Gly Gly
20

INFORMATION FOR SEQ ID NO:87:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

Gly Gly Val Tyr Ala Cys Arg Met Gly Pro Ile Thr Trp Val Cys Ser
1 5 10 15

Pro Leu Gly Gly
20

- 36 -

INFORMATION FOR SEQ ID NO:88:

- (i) SEQUENCE CHARACTERISTICS:

 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: peptide

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

Gly Gly Tyr Thr Thr Cys Arg Met Gly Pro Ile Thr Trp Val Cys Ser
 1 5 10 15

Ala His Gly Gly
20

INFORMATION FOR SEQ ID NO:89:

- (i) SEQUENCE CHARACTERISTICS:

 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii). MOLECULE TYPE: peptide

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

Gly Gly Thr Tyr Lys Cys Trp Met Gly Pro Met Thr Trp Val Cys Arg
 1 5 10 15

Pro Val Gly Gly
20

INFORMATION FOR SEQ ID NO:90:

- (i) SEQUENCE CHARACTERISTICS:

 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: peptide

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i.) SEQUENCE DESCRIPTION: SEQ ID NO:90:

Gly Asn Tyr Tyr Cys Arg Phe Gly Pro Ile Thr Phe Glu Cys His
5 10 15
Thr Gly Gly
20

FORMATION FOR SEQ ID NO:91:

i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

.ii) MOLECULE TYPE: peptide

:i) SEQUENCE DESCRIPTION: SEQ ID NO:91:

Gly Gly Glu Tyr Leu Cys Arg Met Gly Pro Asn Thr Trp Val Cys Thr
1 5 10 15
Pro Val Gly Gly
. 20.

NFORMATION FOR SEQ ID NO:92:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

:xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

Gly Gly Leu Tyr Thr Cys Arg Met Gly Pro Ile Thr Trp Val Cys Leu
1 5 10 15
Pro Ala Gly Gly
20

INFORMATION FOR SEQ ID NO:93:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid

- 88 -

- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

.ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

Gly Gly Leu Tyr Thr Cys Arg Met Gly Pro Ile Thr Trp Val Cys Leu
 1 5 10 15

Pro Ala Gly Gly
20

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- (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

Thr Asn Gly Gly
20

INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

Gly Gly Met Tyr Tyr Cys Arg Met Gly Pro Met Thr Trp Val Cys Lys
 1 . . 5 10 15

Gly Ala Gly Gly
20

INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

Ala Arg Gly Gly
20

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FORMATION FOR SEQ ID NO:72:

- i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

iii) SEQUENCE DESCRIPTION: SEQ ID NO:72:

Gly Pro Tyr His Cys Arg Met Gly Pro Ile Thr Trp Val Cys Gly
5 10 15
Pro Val Gly Gly
20

FORMATION FOR SEQ ID NO:73:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

Gly Gly Glu Tyr Arg Cys Arg Met Gly Pro Ile Ser Trp Val Cys Ser
1 5 10 15
Pro Gln Gly Gly
20

FORMATION FOR SEQ ID NO:74:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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SEQUENCE DESCRIPTION: SEQ ID NO:74:

y Gly Asn Tyr Thr Cys Arg Phe Gly Pro Leu Thr Trp Glu Cys Thr
5 10 15

o Gln Gly Gly Gly Ala
20

FORMATION FOR SEQ ID NO:75:

- i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

iii) SEQUENCE DESCRIPTION: SEQ ID NO:75:

ly Gly Ser Trp Asp Cys Arg Ile Gly Pro Ile Thr Trp Val Cys Lys
5 10 15

rp Ser Gly Gly
20.

FORMATION FOR SEQ ID NO:76:

- iv) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

v) MOLECULE TYPE: peptide

vi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

Val Gly Asn Tyr Met Cys His Phe Gly Pro Ile Thr Trp Val Cys Arg
1 5 10 15

Pro Gly Gly Gly
20

FORMATION FOR SEQ ID NO:77:

- vii) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid

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1

WE CLAIM:

5 1. A peptide dimer comprising two monomeric peptides of 10 to about 40 amino acids in length that bind to EPO receptor, each monomeric peptide comprising a sequence of amino acids X₃X₄X₅GPX₆TWX₇X₈ (SEQ ID NO: 1) wherein X₆ is selected from any of the 20 genetically coded L-amino acids; X₃ is C; X₄ is R, H, L or W; X₅ is M, F or I; X₇ is D, E, I, L or V; and X₈ is C.

10

15 2. The peptide dimer of Claim 1 wherein each of said monomeric peptides comprise a sequence of amino acids YX₂X₃X₄X₅GPX₆TWX₇X₈, (SEQ ID NO: 2) wherein each of X₂ and X₆ is independently selected from any one of the 20 genetically coded L-amino acids; X₃ is C; X₄ is R, H, L or W; X₅ is M, F or I; X₇ is D, E, I, L or V; and X₈ is C.

20

25 3. The peptide dimer of Claim 2 wherein each of said monomeric peptides comprise a sequence of amino acids X₁YX₂X₃X₄X₅GPX₆TWX₇X₈X₉X₁₀X₁₁ (SEQ ID NO: 3) wherein each of X₁, X₂, X₆, X₉, X₁₀, and X₁₁ is independently selected from any one of the 20 genetically coded L-amino acids; X₁ is C; X₄ is R, H, L or W; X₅ is M, F or I; X₇ is D, E, I, L or V; and X₈ is C.

30

35 4. The peptide dimer of Claim 3 wherein X₄ is R or H; X₅ is F or M; X₆ is I, L, T, M or V; X₇ is D or V; X₉ is G, K, L, Q, R, S, or T; and X₁₀ is A, G, P, R, or Y.

40

45 5. The peptide dimer of Claim 4 wherein X₁ is D, E, L, N, S, T or V; X₂ is A, H, K, L, M, B, or T; X₄ is R or H; X₉ is K, R, S, or T; and X₁₀ is P.

50

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6. The peptide dimer of Claim 1 wherein said monomeric peptides are

GGLYLCRFGPVTDGYKGG (SEQ ID NO: 7);
GGTYSCHFGPLTWVCKPQGG (SEQ ID NO: 8);
GGDYHCRMGPLTWVCKPLGG (SEQ ID NO: 9);
VGNYMCHFGPITWVCRPGGG (SEQ ID NO: 10);
GGVYACRMGPITWVCSPPLGG (SEQ ID NO: 11);
VGNYMAHMGPITWVCRPGG (SEQ ID NO: 12);
GGTYSCHFGPLTWVCKPQ (SEQ ID NO: 13);
GGLYACHMGPMTWVCQPLRG (SEQ ID NO: 14);
TIAQYICYMGPETWECRPSPKA (SEQ ID NO: 15);
YSCHFGPLTWVCK (SEQ ID NO: 16);
YCHFGPLTWVC (SEQ ID NO: 17); and
SCHFGPLTWVCK (SEQ ID NO: 18)

7. A pharmaceutical composition comprising at least one peptide dimer of any one of Claims 1-6.

8. A method for treating a patient having a disorder characterized by a deficiency of EPO or low or defective red blood cell population comprising administering to said patient a therapeutically effective amount of at least one peptide dimer of any one of Claims 1-6.

9. The peptide dimer of any one of Claims 1-6 wherein said dimer is formed by a polyethylene glycol linker through a covalent bond.

10. The peptide dimer of any one of Claims 1-6 wherein said monomeric peptide units are dimerized on activated benodiazepins, oxazalones, azalactones, aminimides or diketopiperazine.

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- 1 11. The peptide dimer of Claim 9 wherein said monomeric peptides are covalently bound N-terminus to N-terminus.
- 5 12. The peptide dimer of Claim 10 wherein said monomeric peptides are covalently bound N-terminus to N-terminus.
- 10 13. The peptide dimer of Claim 9 wherein said monomeric peptides are covalently bound N-terminus to C-terminus.
- 15 14. The peptide of Claim 10 wherein said monomeric peptides are covalently bound N-terminus to C-terminus.
- 20 15. A method of improving the bioactivity of a cell surface receptor comprising dimerizing a monomeric agonist of said cell surface receptor and contacting said formed dimer with said cell surface receptor to effect said improved biological activity.
- 25 16. A method of activating a cell surface receptor to induce biological activity of said cell surface receptor comprising dimerizing a monomeric agonist of said cell surface receptor and contacting said formed dimer with said receptor thereby inducing said biological activity.
- 30 17. The method of Claim 15 or 16 wherein said cell surface receptor is contacted with said dimer in vitro or in vivo.
- 35 18. The method of Claim 15 or 16 wherein said cell surface receptor is EPO-R.
- 35 19. The method of Claim 15 or 16 wherein said cell surface agonist is a GH agonist, PDGF agonist, IGF agonist, G-CSF agonist, TPO agonist, VEGF agonist, FGF

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agonist, insulin agonist, IL-3 agonist, IL-5 agonist, IL-6 agonist or IL-2 agonist.

20. The method of Claim 15 or 16 wherein said agonist comprises a sequence of amino acids
5 $YX_2X_3X_4X_5GPX_6TWX_7X_8$, (SEQ ID NO: 2) wherein each of X_2 and X_8 is independently selected from any one of the 20 genetically coded L-amino acids; X_3 is C; X_4 is R, H, L or W; X_5 is M, F or I; X_7 is D, E, I, L or V; and X_8 is C.

10 21. The method of Claim 15 or 16 wherein said agonist comprises a sequence of amino acids
 $X_1YX_2X_3X_4X_5GPX_6TWX_7X_8X_9X_{10}X_{11}$ (SEQ ID NO: 3) wherein each of X_1 , X_2 , X_3 , X_4 , X_5 , X_9 , X_{10} , and X_{11} is independently selected from any one of the 20 genetically coded L-amino acids; X_3 is C; X_4 is R, H, L or W; X_5 is M, F or I; X_7 is D, E, I, L or V; and X_8 is C.

15 22. The method of Claim 15 or 16 wherein said agonist comprises a sequence of amino acids
 $X_1YX_2X_3X_4X_5GPX_6TWX_7X_8X_9X_{10}X_{11}$ (SEQ ID NO: 3) wherein each of X_1 , X_2 and X_{11} is independently selected from any one of the 20 genetically coded L-amino acids; X_3 is C; X_4 is R or H; X_5 is F or M; X_6 is I, L, T, M or V; X_7 is D or V; X_8 is G, K, L, Q, R, S, or T; and X_{10} is A, G, P, R, or Y.

25 23. The method of Claims 15 or 16 wherein said agonist comprises a sequence of amino acids
 $X_1YX_2X_3X_4X_5GPX_6TWX_7X_8X_9X_{10}X_{11}$ (SEQ ID NO: 3) wherein X_1 is D, E, L, N, S, T or V; X_2 is A, H, K, L, M, S, or T; X_3 is C; X_4 is R or H; X_5 is M, F or I; X_6 and X_{11} are independently any one of the 20 genetically coded L-

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1 amino acids; X₁ is D, E, I, L or V; X₂ is C; X₃ is K, R, S, or T; and X₁₀ is P.

5 24. The method of Claim 15 or 16 wherein said agonist is selected from the group consisting of:

GGLYLCRFGPVTDGYKGG (SEQ ID NO: 7);
GGTYSCHFGPLTWVCKPQGG (SEQ ID NO: 8);
GGDYHCRMGPLTWVCKPLGG (SEQ ID NO: 9);
VGNYMCHFGPITWVCRPCGG (SEQ ID NO: 10);
GGVYACRMGPITWVCSPPLGG (SEQ ID NO: 11);
VGNYMAHMGPITWVCRPCGG (SEQ ID NO: 12);
GGTYSCHFGPLTWVCKPQ (SEQ ID NO: 13);
GGLYACHMGPMTWVCQPLRG (SEQ ID NO: 14);
TIAQYICYMGPETWECRPSPKA (SEQ ID NO: 15);
YSCHFGPLTWVCK (SEQ ID NO: 16); and
15 YCHFGPLTWVC (SEQ ID NO: 17).

20 25. The method of Claim 15 or 16 wherein said peptide dimers are formed with a polyethylene glycol linker through a covalent bond.

26. A method of preparing a cell surface receptor agonist comprising dimerizing a cell surface antagonist.

25 30 35. The method of Claim 26 wherein said cell surface antagonist receptor is a GH antagonist, PDGF antagonist, EGF antagonist, G-CSF antagonist, EGF antagonist, GM-CSF antagonist, TPO antagonist, VEGF antagonist, FGF antagonist, insulin antagonist, IL-3 antagonist, IL-5 antagonist, IL-6 antagonist, or an IL-2 antagonist.

30 35. The method of Claim 26 wherein said cell surface receptor antagonist is a EPO-R antagonist.

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1 29. The method of Claim 28 wherein said
antagonist comprises a sequence of amino acids
 $(AX_2)_nX_3X_4X_5GPX_6TWX_7X_8$ (SEQ ID NO: 19) wherein X_6 is
selected from any of the 20 genetically coded L-amino
5 acids; X_3 is C; X_4 is R, H, L or W; X_5 is M, F or I; X_7
is D, E, I, L or V; X_8 is C; X_2 is selected from any of
the 20 genetically coded L-amino acids, n is 0 or 1 and
A is any of the 20 genetically coded L-amino acids
except Y (tyrosine).

10 30. The method of Claim 21 where said
antagonist is SCHFGPLTWVCK (SEQ ID NO: 18).

15

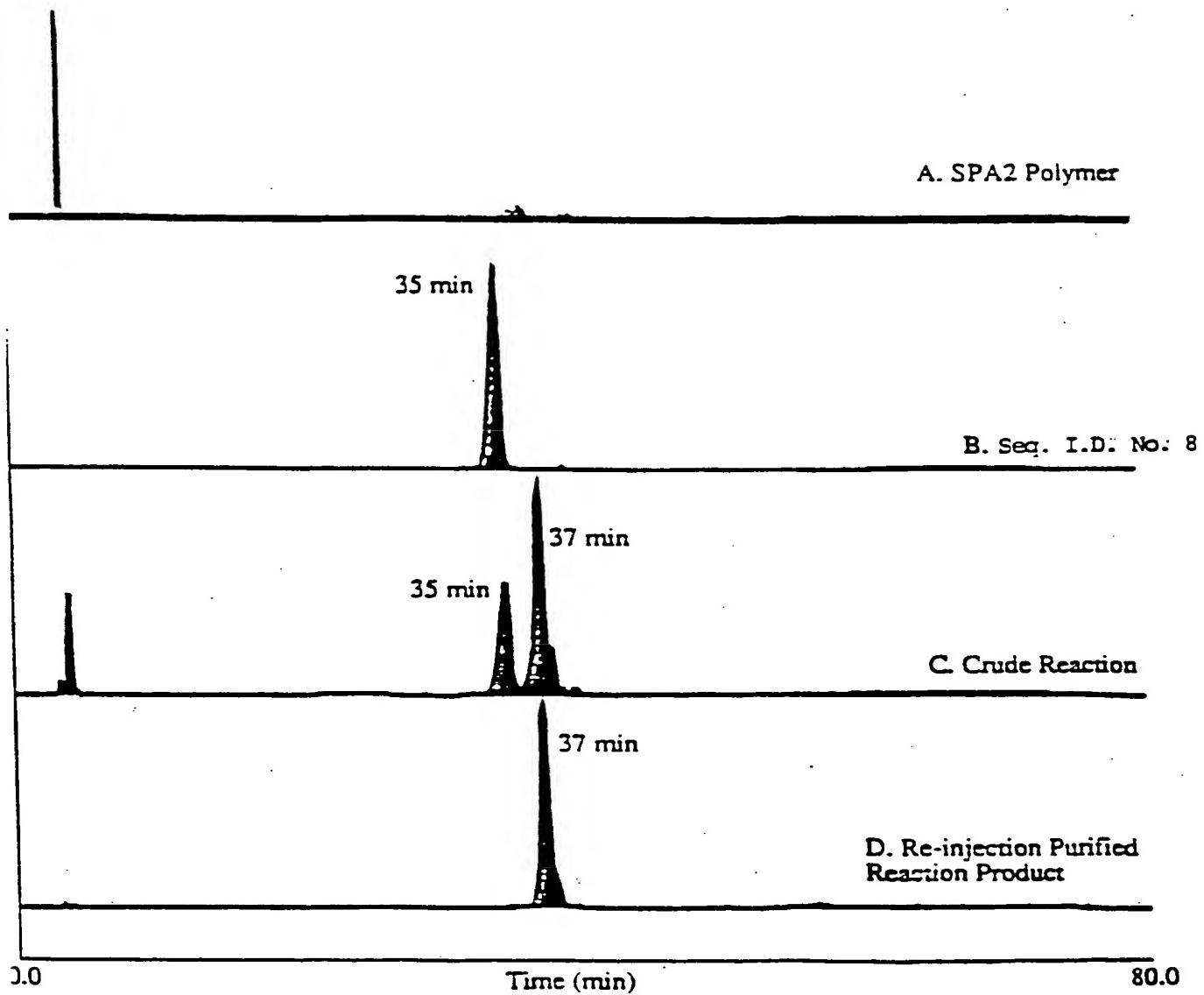
20

25

30

35

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Reverse Phase Analysis of SPA2 Reaction with SEQ. I.D. NO. 8**Figure 1**

Preparative Reverse Phase Analysis of PEG-Peptide (SEQ. I.D. No. 8) Dimer

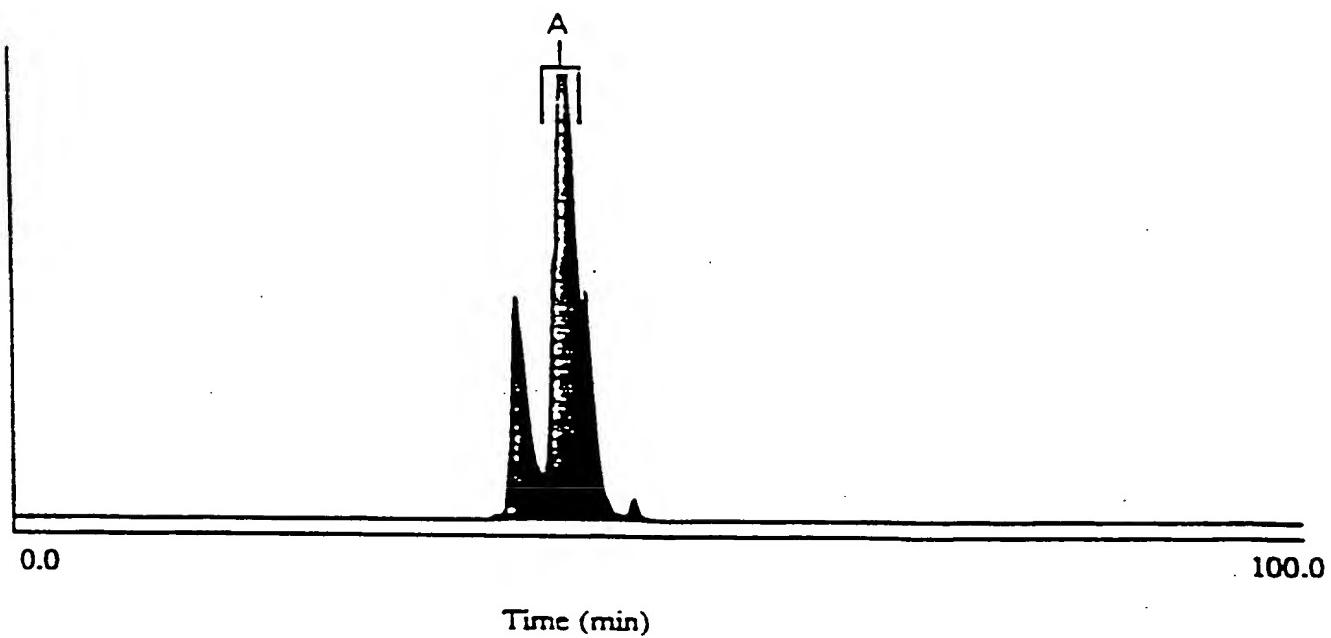
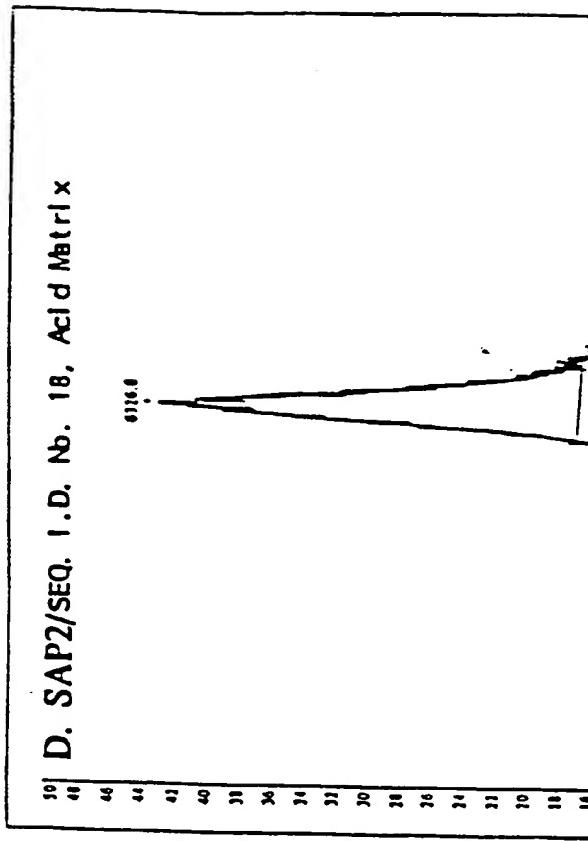
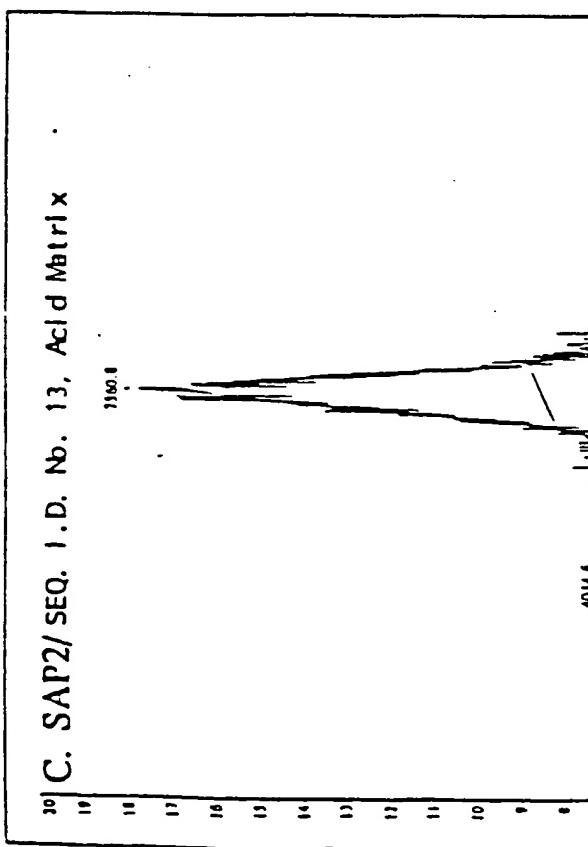
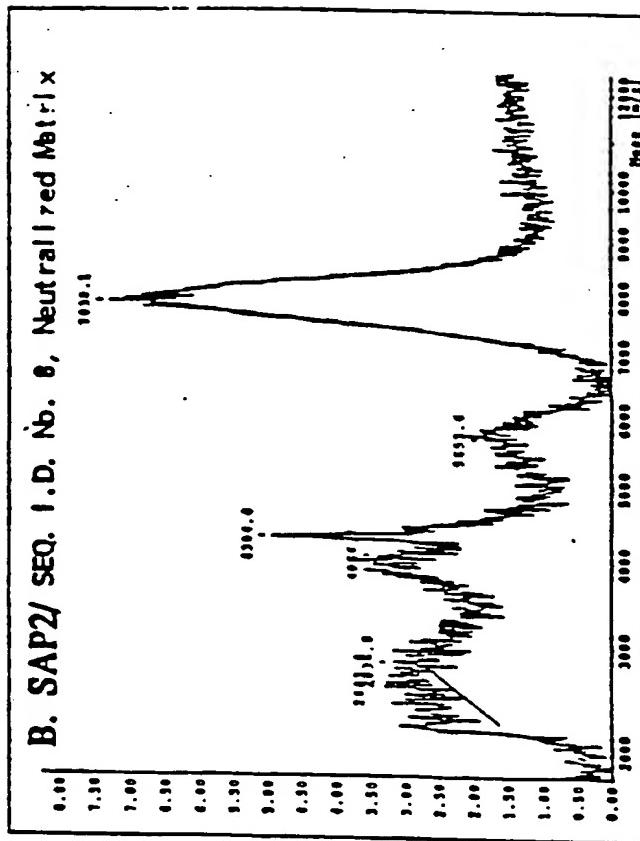
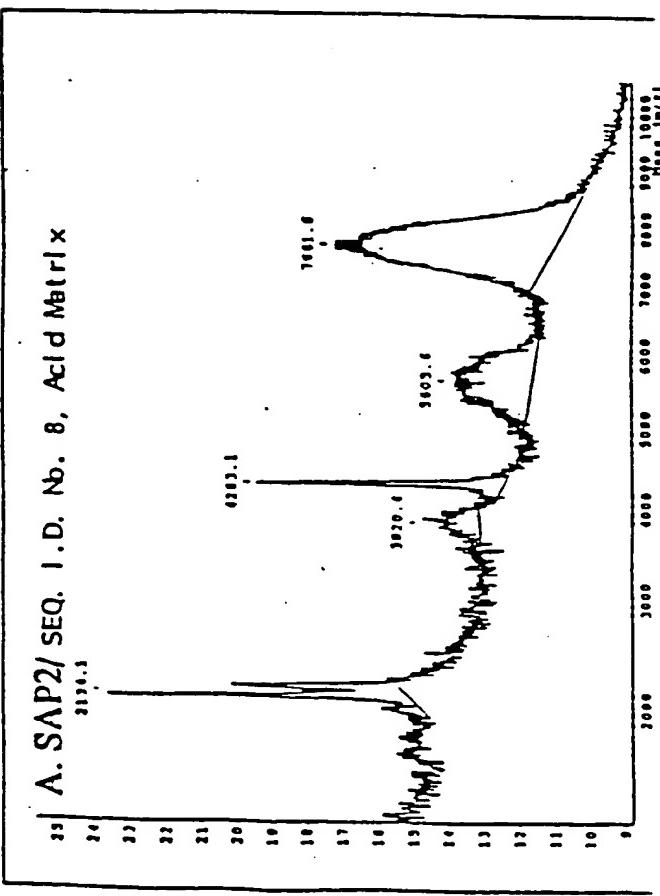
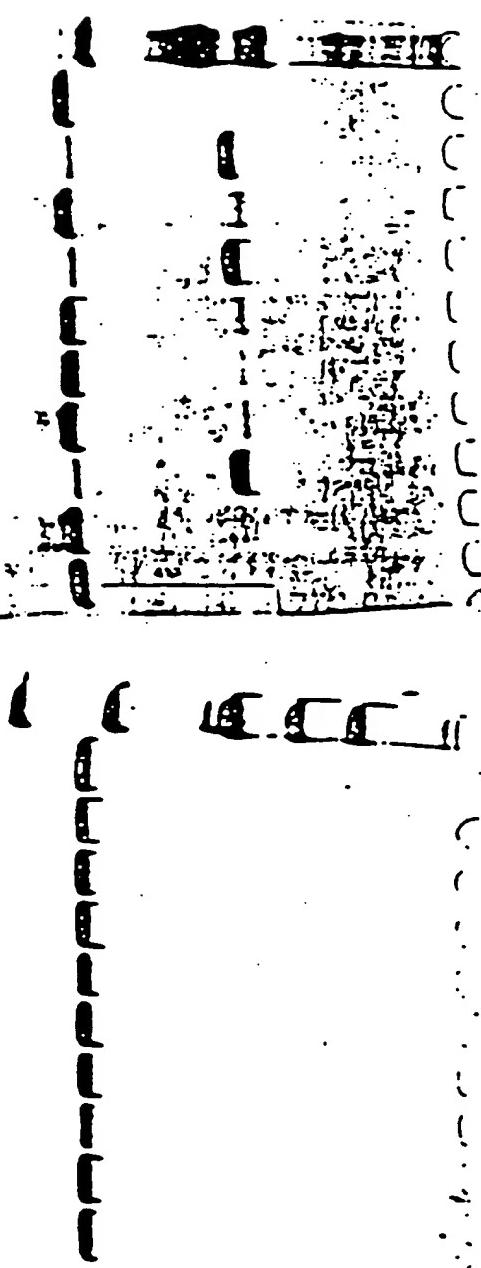


Figure 2

Figure 3



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A. Non Reducing 10-20% SDS-PAGE, B. Reducing 10-20% SDS-PAGE

Lane	Seq. I. D. No.	8	16	18	13					
1	2	3	4	5	6	7	8	9	10	11
MW	EBP	Peptide (μ M)	400	400	400	400	400	400	400	...
Ern (pM)	22	22	22	22	22	22	22	22	22	22
DPCP (mM)	1.1	0	1.1	0	1.1	0	1.1	0	1.1	0

Seq. I. D. No.	Sequence	[C] ₅₀ (μ M)	ERD-ED ₅₀ (μ M)
8	GCTYSCLIFGRLTMVCKPQCG	5	0.1
16	YSCLIFGRLTMVCK	70	3
18	SCIFGRLTMVCK	90	1A
13	GCTYSCLIFGRLTMVCKPQ	8	0.08

Figure 4

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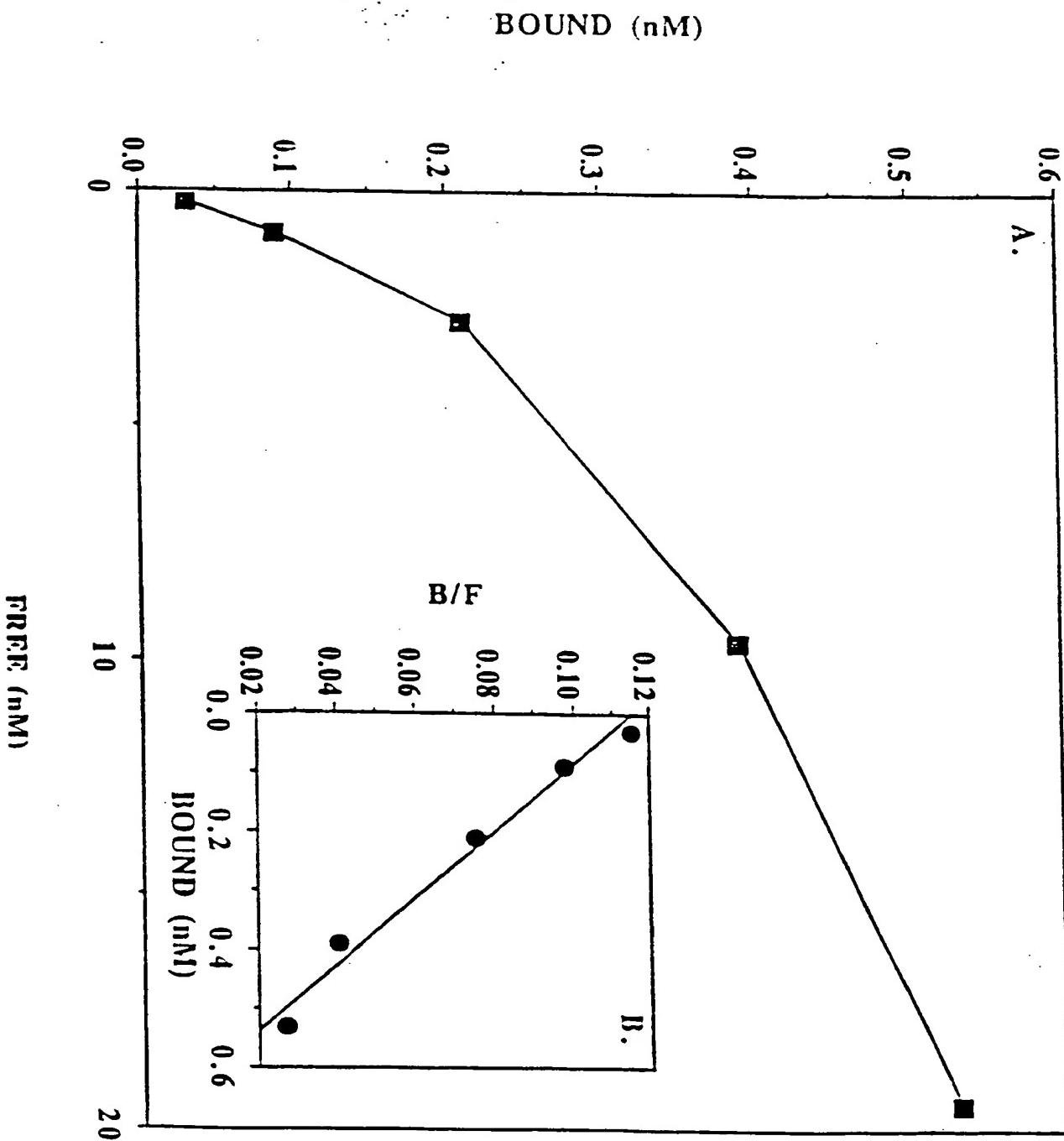
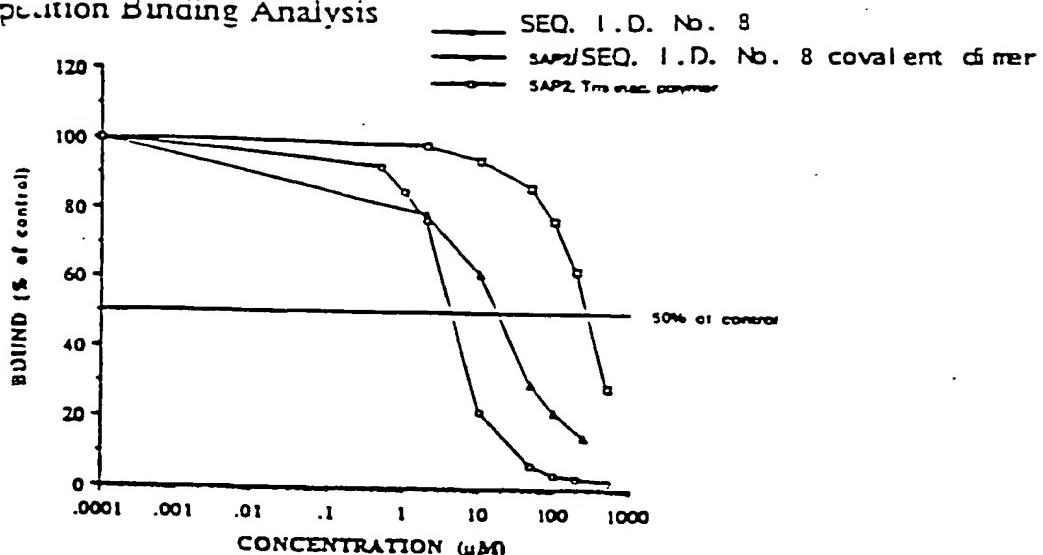
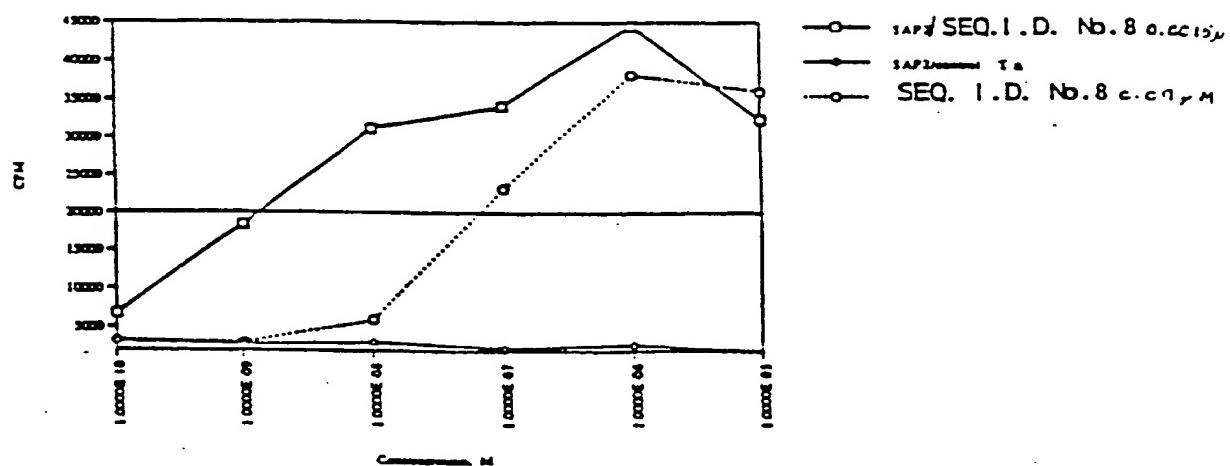


Figure 5

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A. [¹²⁵I]EPOR Competition Binding Analysis

B. Cell Proliferation, Human EPOR



C. Cell Proliferation, Murine EPOR

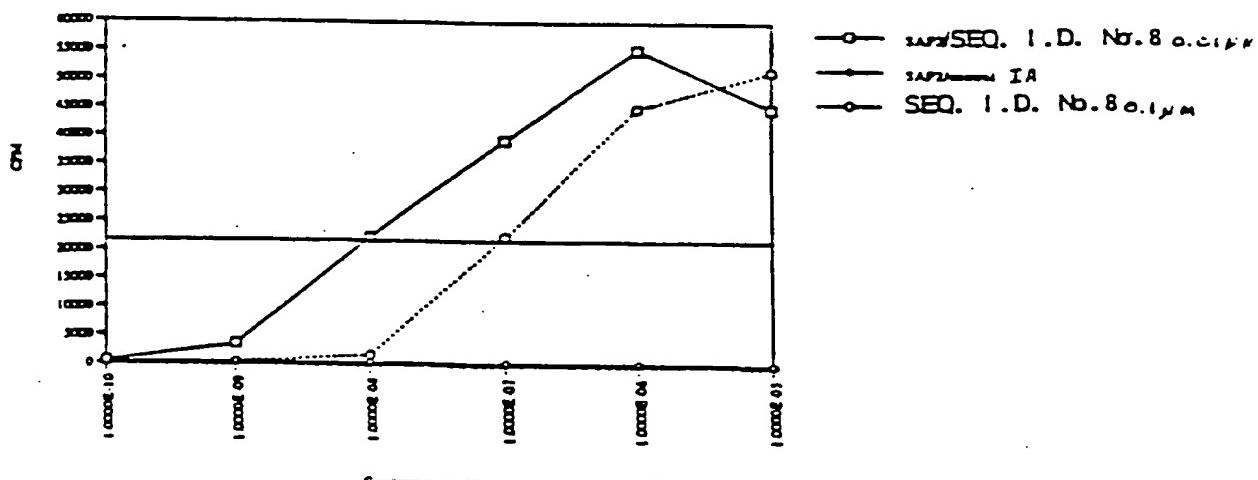


Figure 6

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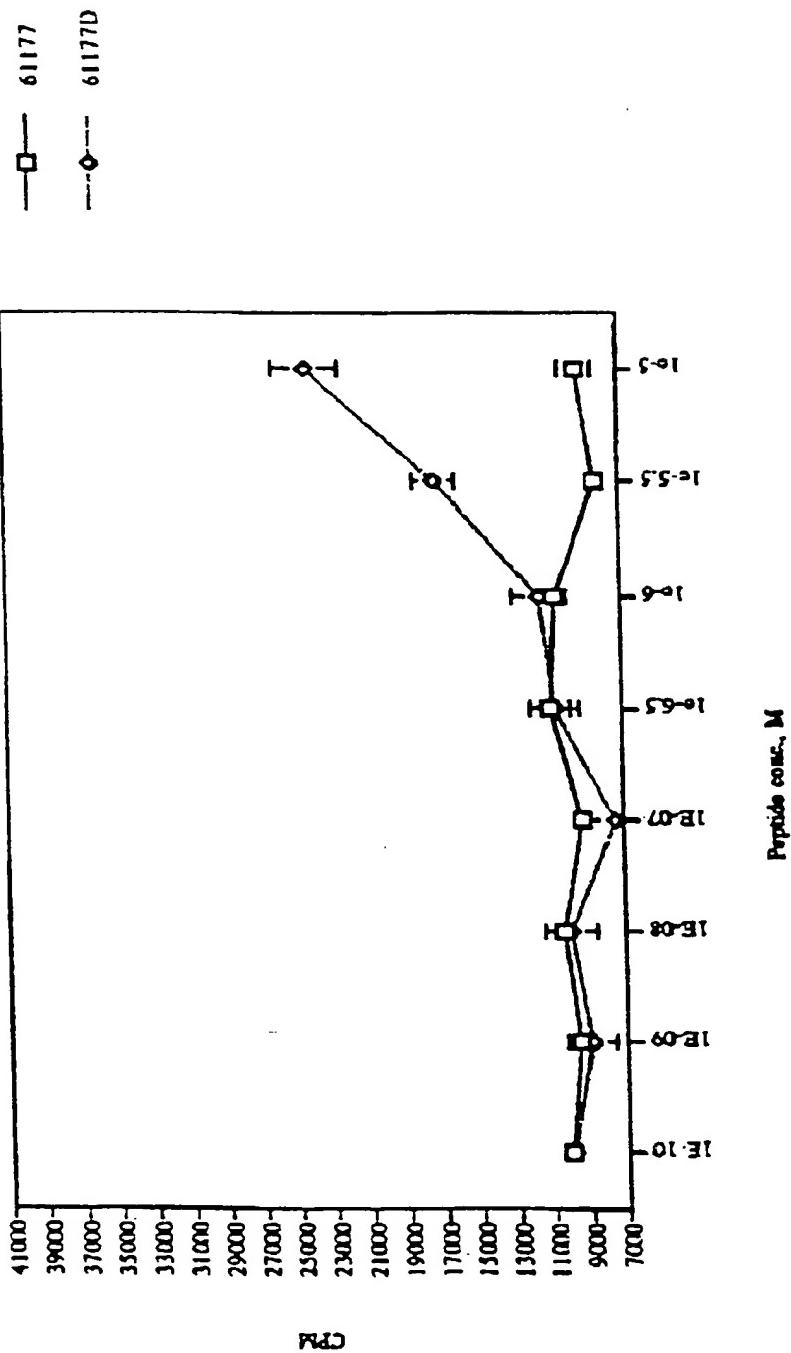


Figure 6
Panel D

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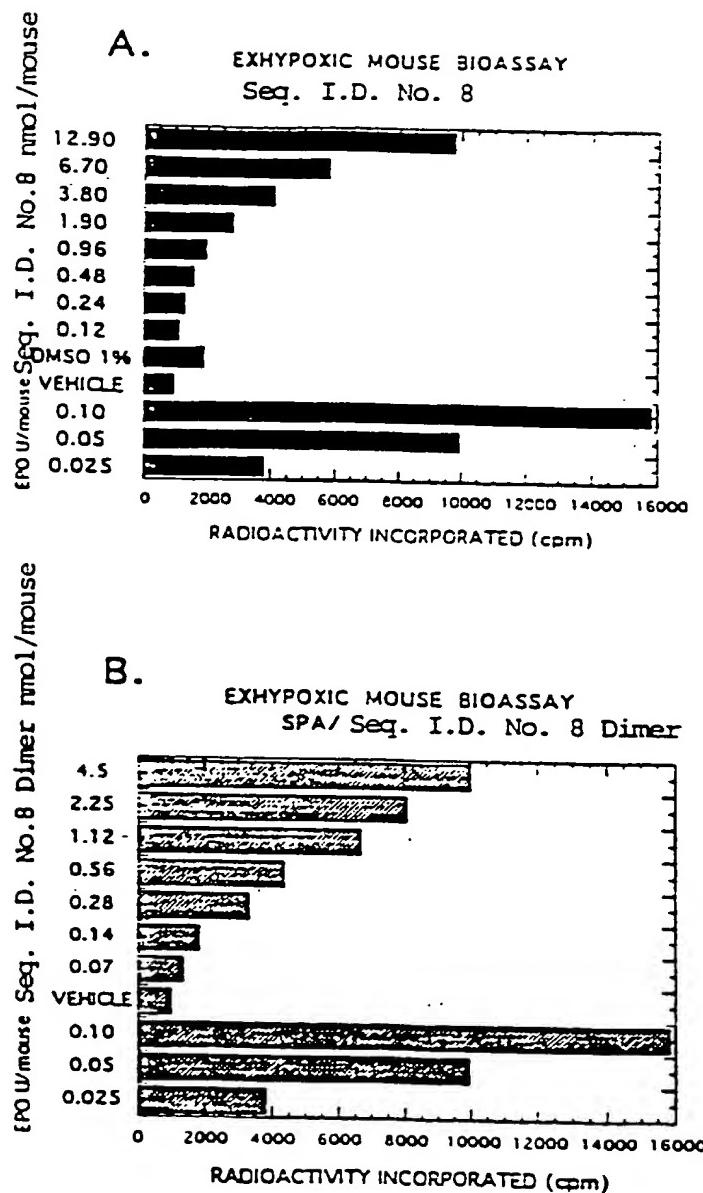


Figure 7

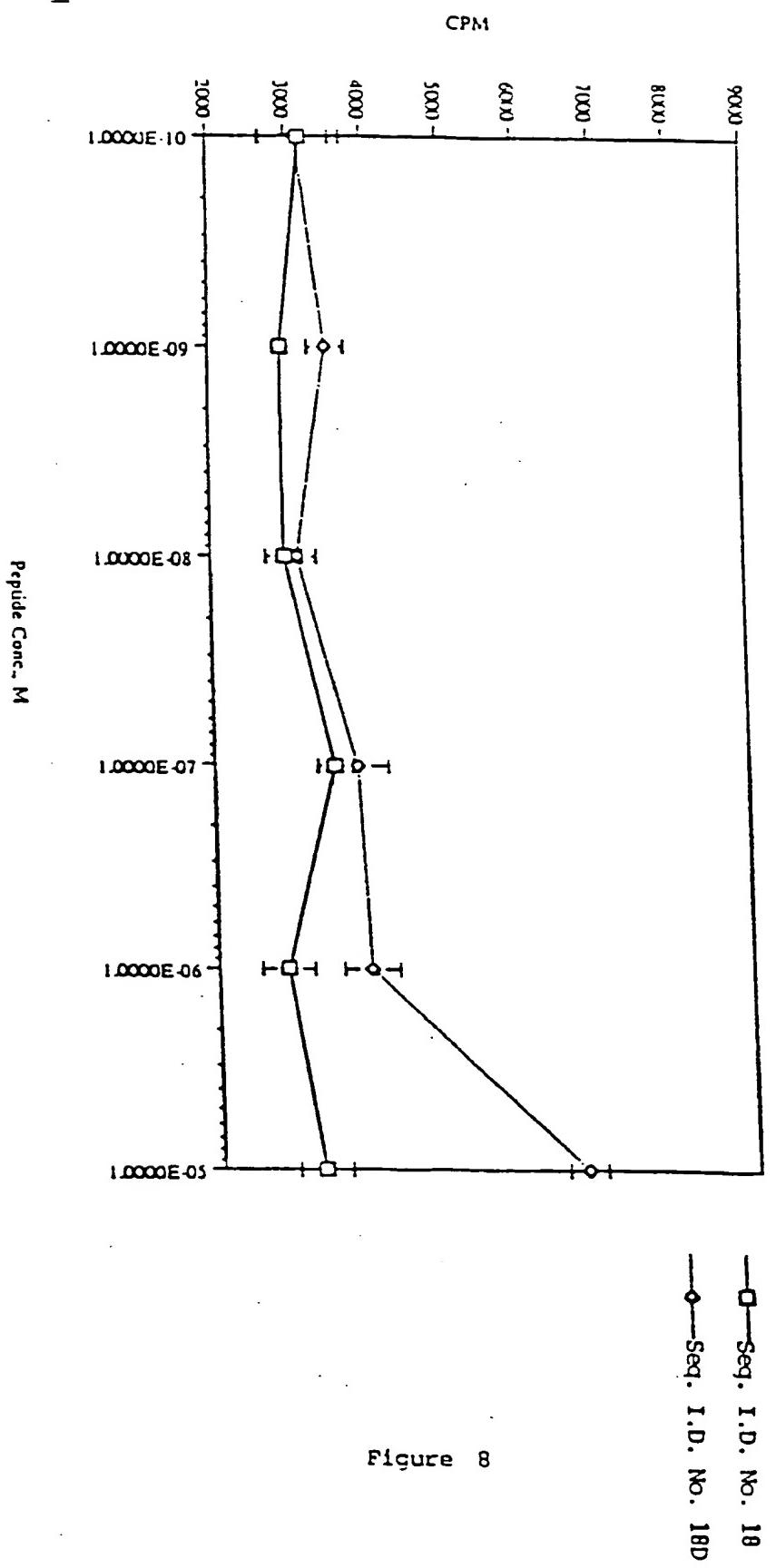


FIGURE 9

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Ac-GGTYSGHFGPLTWVCKPQGG	SEQ ID NO. 20
GCGTTCAGCCTGATTTTACGAGG	SEQ ID NO. 21
GGTAACTTGCTGATTAACGAGG	SEQ ID NO. 22
GGTAACTTGCTGATTAACGAGG	SEQ ID NO. 23
GGTAACTTGCTGATTAACGAGG	SEQ ID NO. 24
GGTAACTTGCTGATTAACGAGG	SEQ ID NO. 25
GGTAACTTGCTGATTAACGAGG	SEQ ID NO. 26
GGNYLCRFGPATWVCKPQGG	SEQ ID NO. 27
GGNYVCRCMGPITWVCKPQGG	SEQ ID NO. 28
GGSYLSCRMGPITWVCKPQGG	SEQ ID NO. 29
GGSYLSCRMGPITWVCKPQGG	SEQ ID NO. 30
GGSYLSCRMGPITWVCKPQGG	SEQ ID NO. 31
GGSYLSCRMGPITWVCKPQGG	SEQ ID NO. 32
GGSYLSCRMGPITWVCKPQGG	SEQ ID NO. 33
GGSYLSCRMGPITWVCKPQGG	SEQ ID NO. 34
GGSYLSCRMGPITWVCKPQGG	SEQ ID NO. 35
GGSYLSCRMGPITWVCKPQGG	SEQ ID NO. 36
GGSYLSCRMGPITWVCKPQGG	SEQ ID NO. 37
GGSYLSCRMGPATWVCKPQGG	SEQ ID NO. 38
GGSYLCRFGPVLTWDCGTYGG	SEQ ID NO. 39
GGSYLSCRMGPITWVCKPQGG	SEQ ID NO. 40
GGSYLSCRMGPMTWVCRPVGG	SEQ ID NO. 41
GGTYSGHFGPLTWVCKPQGG	SEQ ID NO. 42
GGTYSGHFGPLTWVCKPQGG	SEQ ID NO. 43
GGYSCRMGPITWLCXPREG	SEQ ID NO. 44
GGYTHCRMGPITWVCRPLGG	SEQ ID NO. 45
GGYSCRMGPITWVCTPREG	SEQ ID NO. 46
GGYTHCRMGPSTWEDNAHGG	SEQ ID NO. 47
GGYLCSTFGPATWVCKPQGG	SEQ ID NO. 48
GGYKCRRLGPATWVCSPLGG	SEQ ID NO. 49
GGSYLSCRMGPETWVCRPNGG	SEQ ID NO. 50
GGSYLSCRMGPITWVCKPQGG	SEQ ID NO. 51
GGSYLSCRMGPITWVCLPAGG	SEQ ID NO. 52
GGYLGSCRMGPMTWVCRPGGG	SEQ ID NO. 53

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Figure 9
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GGDYTCRNGPITWICTKAGG	SEQ ID NO: 54
GGVYSCRNGPTT.WCNPRYVG	SEQ ID NO: 55
GGAYLCRNGPIT.WCRPQGG	SEQ ID NO: 56
GGEYSCRNGPNTWVCKPVGG	SEQ ID NO: 57
GGLYLCRNGPVT.WECOPRGCG	SEQ ID NO: 58
GGLYTCRNGPITAVCLLPGG	SEQ ID NO: 59
GGLYTCRNGPVT.WVCTGAGG	SEQ ID NO: 60
GGJYKCRNGPLT.WECRPTGG	SEQ ID NO: 61
GGDYNCRFCPLT.WCKPSGG	SEQ ID NO: 62
GGSYLCRNGPTT.WLSSXGG	SEQ ID NO: 63
GGSYLCRNGPTT.WCTPMGG	SEQ ID NO: 64
GGSYLCRFCPTT.WLCTORGCG	SEQ ID NO: 65
GGWVTCRNGPIT.WCGVHGG	SEQ ID NO: 66
GGQLLCIGCPTT.WCRVNGG	SEQ ID NO: 67
GGKYSFCRNGPTT.WCSPVGRGV	SEQ ID NO: 68
GGAVYCRICPIT.WVCDTRGG	SEQ ID NO: 69
GGMYYCRNGPMTHVCKGAGG	SEQ ID NO: 70
GGTTOCHICPITWVCRARGG	SEQ ID NO: 71
GGPYHCRNGPIT.WVCPVGG	SEQ ID NO: 72
GGEYRCRNGPISWCSPOGGC	SEQ ID NO: 73
GGMYTCRFCPLTWECTPOGGCA	SEQ ID NO: 74
GGSHDCRICPITAVCKHSGG	SEQ ID NO: 75
VQNYMCHFGPITWVCRPGGG	SEQ ID NO: 76
GGLYLCRNGPOTWNCOPGGG	SEQ ID NO: 77
GGDYVCRNGPMTWVCAHYGR	SEQ ID NO: 78
GGWYSCLMGPMTWVCKAHRG	SEQ ID NO: 79
GGKYYCWNGPMTWVCSPAGE	SEQ ID NO: 80
GGYVMCRICPITWVCDIPGG	SEQ ID NO: 81
GSTLOCCICGPITWVCRHAGG	SEQ ID NO: 82
GGEYFRCYGCPTT.WTCSEJ	SEQ ID NO: 83
GGEYICRNGPLT.WECKRTGG	SEQ ID NO: 84
GGLYACRNGPITWVKYMAAG	SEQ ID NO: 85
GGOYLCTFCGPITWLCRAGGG	SEQ ID NO: 86
GGVYACRNGPITHVCSPLGC	SEQ ID NO: 87
GGYTTCRNGPITWVCSAHGG	SEQ ID NO: 88
GGTYKCHNGPMTWVCRPVGC	SEQ ID NO: 89
GGNYYCRFCGPITFECHPTCC	SEQ ID NO: 90

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GGEYLC?MG?MT..VCT?VCC	SEQ ID NO: 91
GGLYTCA?MG?IT..VCLPAGC	SEQ ID NO: 92
GGLYTCA?MG?IT..VCLPAGC	SEQ ID NO: 93

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